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FIELD RELEASES AND EVALUATION OF NEW BIOLOGICAL CONTROL AGENTS FOR SCENTLESS CHAMOMILE

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FIELD RELEASES AND EVALUATION OF BIOLOGICAL CONTROL AGENTS FOR SCENTLESS CHAMOMILE

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ABSTRACT

Four European insect biological control agents for scentless chamomile were studied to evaluate methods of rearing and field release, assess their survival and establishment in the field, and evaluate their impact on the weed. The gall midge *Rhopalomyia tripleurospermi* was approved for release in 1999. A successful greenhouse rearing method was developed, and numerous releases have resulted in establishment in Alberta and Saskatchewan. Releases can be made by transplanting infested plants into field populations of scentless chamomile. The gall midge is spreading rapidly, and completes three generations in most growing seasons in central Alberta. The stem-mining weevil *Microplontus edentulus* is confirmed to be established at one site each in Alberta and Saskatchewan, and may be established at one other site in each province. Difficulties in mass rearing have limited the number of releases of the stem weevil that could be made. Establishment was most successful when infested plants were transplanted into a field site. If releases of adult stem weevils are to be made, the chances of establishment may be increased by using field cages initially to limit dispersal. The seed weevil *Omphalapion hookeri* has continued to spread from established release sites in Alberta and Saskatchewan. In two field plot experiments to evaluate the effects of all three established insect species, plants in all treatments became very large and vigorous, masking most effects of the insects. The gall midge produced significant height reductions in scentless chamomile in two experiments, and the seed weevil reduced seed weight. Further evaluation of the impact of these insects on scentless chamomile should focus on their effects on its ability to compete with crops or other vegetation. A fourth insect, the stem mining fly *Napomyza* sp. near *lateralis*, was subjected to host specificity and biological studies in Europe. Although it is fairly specific to scentless chamomile, it was decided not to seek approval for its release in Canada due to concerns about possible attack on some minor horticultural crops and uncertainty about its taxonomic status.

1. BACKGROUND INFORMATION

1.1. The scentless chamomile problem

Scentless chamomile (*Matricaria perforata* Mérat, *Tripleurospermum inodorum* (L.) Schultz Bip., Asteraceae) is a plant of European origin which has become naturalized in Canada, and is now a serious weed of disturbed and agricultural land in the prairie provinces. Scentless chamomile occurs widely in the black, grey and dark brown soil zones of Alberta and Saskatchewan, and can spread rapidly because of its profuse seed production. It occurs in a wide variety of habitats, including annual and perennial crops, pastures, wasteland, roadsides and ditches (Woo et al. 1991). Scentless chamomile can become abundant in urban or industrial areas or along pipeline and utility rights-of-way, forming a reservoir for further infestation of agricultural land. Scentless chamomile can cause significant yield losses by competing with cereal crops (Douglas et al. 1991; Douglas et al. 1992).

1.2. Biology and life history of scentless chamomile

Scentless chamomile is an annual or short-lived perennial. Plants germinating before mid-July usually show an annual life cycle, bolting and flowering within the same growing season, while plants germinating from mid-July onwards usually follow the winter annual life cycle, developing onto an overwintering rosette which bolts and flowers the following summer (Blackshaw and Harker 1997). Most plants die after flowering and setting seed, but a small percentage may survive over winter and regrow from the base to flower again in the following season (short-lived perennial life cycle). In central Alberta, scentless chamomile usually begins flowering in early to mid July and seed is set from early August to freeze-up. Dense, vigorous populations of scentless chamomile can produce up to 1.8 million seeds m^{-2} (Woo et al. 1991)

1.3. Previous biological control work

In Canada scentless chamomile is attacked by few insects or diseases, almost all of which are generalists (Woo et al. 1991). In Europe, in contract, it is attacked by numerous natural enemies, some of them specific (Freese and Günther 1991). Scentless chamomile was therefore proposed as a candidate for biological control in Canada (Peschken 1989; Peschken et al. 1990). A seed-feeding weevil, *Omphalapion hookeri*, has been introduced from Europe and has established well at several sites in Alberta and other prairie provinces (McClay and De Clerck-Floate 1999) from material imported both from Europe and from an adventive population in Nova Scotia.

Two other European insects have been screened and approved for field release in Canada under the CAESA program and AARI Matching Grants program. The stem-mining weevil *Microplontus edentulus* was first released in 1997 and preliminary results indicated that it had survived overwinter and bred at field plots at ARC Vegreville. The gall-forming midge *Rhopalomyia tripleurospermi* was approved for field release in Canada in March 1999. This insect appears particularly promising as it will attack and

form galls on scentless chamomile plants at a variety of different growth stages, and has up to four generations per year in Europe. Screening of another agent, the stem-mining fly *Napomyza* sp. nr. *lateralis* was almost completed at CABI Bioscience in Switzerland.

Although these three insects were available for biological control of scentless chamomile in Canada, there was little information on the best ways to rear, release, or establish them in the field, or on their impacts on scentless chamomile plants or populations. The goal of this project was to test methods of rearing, releasing and monitoring these biological control agents for scentless chamomile, and to evaluate their establishment success and impact on the weed in the field. We also completed tests on two other potential European biological control agents for scentless chamomile.

1.4. Biology of scentless chamomile biocontrol agents

The scentless chamomile seed weevil *Omphalapion hookeri* lays eggs into the young flower buds of scentless chamomile as they begin to open, between the disk florets. When the larvae hatch they feed on the developing seeds, each one consuming about 11 seeds during its development. Numerous weevils can develop within a single flower head. The larvae pupate in the flower head and adults emerge in late summer to feed on scentless chamomile foliage, although the feeding damage is insignificant. The adults overwinter and there is a single generation per year(Freese 1991; Peschken and Sawchyn 1993; McClay and De Clerck-Floate 1999).

The stem weevil *Microplontus edentulus* oviposits into bolting stems of scentless chamomile. The larvae feed in a tunnel inside the stems until mature, when they chew an exit hole at a node through which they crawl out and drop to the soil. They tunnel into the soil and form a cell where they pupate and overwinter. Adults emerge from the soil in the spring. There is a single generation (Hinz et al. 1996; McClay et al. 2002).

The gall midge *Rhopalomyia tripleurospermi* was discovered in Europe during surveys conducted for biocontrol agents. The adults are tiny delicate flies and live only a few hours. Females lay eggs on the growing points of scentless chamomile plants, and the newly hatched larvae tunnel into the meristematic tissue, where they induce the development of a multi-chambered gall. Galls can be formed in terminal or axillary buds, in the centre of rosettes, or on flowers. In Europe *R. tripleurospermi* undergoes up to four generations per year, overwintering as mature larvae in galls (Hinz 1998; Hinz and Müller-Schärer 2000; Skuhravá and Hinz 2000; McClay et al. 2002).

The agromyzid fly *Napomyza* sp. near *lateralis* lays eggs mainly into the midveins of scentless chamomile leaves but also into the stems. Larvae mine in the shoot tips, flower buds, overwintering rosettes, and root crowns. In Europe there are three or four generations per year; overwintering appears to occur as larvae in rosettes (Hinz and Leiss 1996). The systematics of the *Napomyza lateralis* group are not clear. Earlier records suggested that *N. lateralis* would feed on a large number of species in the Asteraceae

(Spencer 1966). However, Spencer (1976) suggested that *N. lateralis* may be a species group probably consisting of relatively specialized sibling species. Scentless chamomile is said to be the main host of this group. The *lateralis* group has been split at present into 39 different species, many of which have very restricted host ranges (Zlobin 1993; Zlobin 1994). Flies morphologically identical to *Napomyza lateralis* have been reared from Russian knapweed, *Acroptilon repens* (Schaffner et al. 1999).

The anthomyiid fly *Botanophila spinosa* was found as mature larvae and pupae in overwintered rosettes of scentless chamomile in Switzerland (Hinz and Leiss 1996). Adult flies have been reared out but the life history is not known. The systematics of this group is also not clear. Most known species of *Botanophila* are restricted to a few closely-related host plants. However, flies identified as *B. spinosa* are recorded attacking *Onopordum* species, and have been introduced into Australia as biocontrol agents for *Onopordum acanthium* (Vitou et al. 2001). This suggests that the name *B. spinosa* is being applied to a complex of related species with different host plants.

2. PROJECT OBJECTIVES

1. To test methods of rearing, releasing and monitoring the stem-mining weevil *Microplontus edentulus* as a biological control agent for scentless chamomile
2. To evaluate the survival of *Microplontus edentulus* in the field and its impact on scentless chamomile.
3. To test methods of rearing, releasing and monitoring the gall midge *Rhopalomyia* n. sp. as a biological control agent for scentless chamomile
4. To evaluate the survival of *Rhopalomyia* n. sp. in the field and its impact on scentless chamomile.
5. To compare the fecundity, survival and feeding rates of the two available populations of *Omphalapion hookeri*.
6. To complete testing and introduction of *Napomyza* sp. nr. *lateralis*.

Objective 5 was dropped due to the lack of availability of material of the Nova Scotia population. The impact evaluation components of objectives 2 and 4 were combined in two experiments, with the addition of *Omphalapion hookeri* in a full factorial design, so that the impact of all currently available insects could be evaluated.

3. MATERIALS AND METHODS

3.1. Rearing, release, and monitoring of *Microplontus edentulus*

Two shipments of *M. edentulus* adults collected in eastern Austria were received from CABI Bioscience Switzerland, on May 19 2000 and June 4 2001. Both shipments arrived in good condition. The 2000 shipment contained 256 ♂ and 581 ♀, and the 2001 shipment contained 110 ♂ and 383 ♀.

Microplontus edentulus was reared by placing adults on scentless chamomile plants in 22-cm diameter pots in an outdoor screened rearing cage. Large plants with multiple bolting stems were used whenever possible. Plants were placed on steel tables with wire mesh tops approximately 1 m above ground level. The mesh size was large enough for emerging larvae to fall readily through the mesh. When larval emergence from stems began, trays were placed under the tables to collect the larvae as they fell. Trays were inspected and larvae collected daily between 8 and 10 a.m. (most larval emergence takes place early in the morning). Larvae were taken into the laboratory and placed on slightly moistened greenhouse soil mixture in plastic boxes 21 × 31 × 9 cm (Rubbermaid "Servin' Saver" 4.2 L). Most larvae rapidly tunnelled into the soil. Up to 300 larvae were placed in each box, which was then covered and stored at 10°C for 14 days and over winter at 0°C. The boxes were brought out into room temperature in late May of the following year and emerging adults were collected. As some larvae would fall into the soil in the pots instead of the collecting trays, the plants used in the rearing cage were cut back at the end of the summer and the pots were stored out of doors until the following spring for collection of emerging adults.

Releases of *M. edentulus* were made into field stands of scentless chamomile, either in screened field cages or in the open. Release sites were monitored by collecting samples of stems (usually 50 stems) in September, after larval emergence, and dissecting the stems to search for larval tunnels, frass, and emergence holes. These were usually easily visible in stems attacked by *M. edentulus*. Sampling was also conducted by sweep netting in patches of scentless chamomile in spring and early summer, and by placing emergence traps (1 m^2 pyramid-shaped screened cages) over patches of scentless chamomile in early spring and searching the cages periodically for emerged adults.

Field releases of *M. edentulus* in British Columbia, Alberta, Saskatchewan and Manitoba are listed in Table 1.

3.2. Rearing, release, and monitoring of *Rhopalomyia tripleurospermi*

Scentless chamomile plants for rearing of *R. tripleurospermi* were usually grown from seed in 10-cm square plastic pots in greenhouse soil mixture and thinned to one plant per pot.

Adult *R. tripleurospermi* were collected from galled scentless chamomile plants in emergence cages in the greenhouse and placed on fresh potted scentless chamomile plants in cages. Usually around 10 female midges were used per plant. Numbers of males were usually somewhat lower as the sex ratio in *R. tripleurospermi* is skewed towards females. Plants used were in the rosette stage whenever possible. Plants were removed from the cages 24 h after exposure to oviposition and left on the greenhouse bench. About 28 days after exposure, if galls were visible, plants were again transferred to emergence cages for collection of adults. For mass rearing, galled plants were

sometimes also interspersed among fresh plants on the greenhouse bench and adults were allowed to emerge, disperse, and oviposit freely in the greenhouse. Galled plants were stored over winter in a cool growth chamber at 6°C to provide material for restarting the rearing process the following spring.

Approval for field releases of *R. tripleurospermi* was received from the Canadian Food Inspection Agency, Plant Health and Production Division, on March 17, 1999. The first releases of *R. tripleurospermi* were made on April 22, 1999, on scentless chamomile plots at ARC, Vegreville. Between this date and June 29, 1,208 female and 630 male *R. tripleurospermi* from the greenhouse colony were released into 1 m^3 cages (20 mesh per inch Saran screening) on five separate locations within the plots.

Further releases of *R. tripleurospermi* at field sites were made by two methods. Newly emerged adults were taken from the greenhouse rearing population and released into 1 m^3 screened cages on field stands of scentless chamomile. Because adult *R. tripleurospermi* live only a few hours, this was only feasible at sites within 120 km of Vegreville. Releases were also made by transplanting galled scentless chamomile plants into field patches of scentless chamomile. Usually nine galled plants were transplanted into the field site in a 3 × 3 grid pattern with 1 m spacing between plants. Sites were monitored by collaborators after transplanting and watered if necessary until the transplants became established. Details of releases made are shown in Table 2.

Release sites of *R. tripleurospermi* were monitored by searching the vicinity of each release site for galls on scentless chamomile plants. If galls were found, the time taken to locate 10 galls was recorded as a measure of gall abundance. At some sites the location of galled plants was recorded using a GPS receiver to estimate rates of dispersal of *R. tripleurospermi* from the release point.

3.3. Impact evaluation

Four field studies were set up on the ARC Vegreville site to evaluate the impact of biological control agents for scentless chamomile. In 2000 and 2001, factorial field plot experiments were set up using all three agents (*O. hookeri*, *M. edentulus*, and *R. tripleurospermi*) in all possible combinations. Two field studies were set up in 2001 to estimate the impact of *R. tripleurospermi* alone on scentless chamomile.

3.3.1. Factorial plot experiment 2000

The first experiment was started in spring 2000. This was a factorial experiment with eight treatments (all possible combinations of presence or absence of the three insect species) and four replicates, in a randomized complete block design (Figure 1). Four scentless chamomile rosettes that had been grown from seed in the greenhouse were transplanted into each of 32 1 m^2 plots on May 1 2000. Plots were spaced 1.5 m apart. On May 5 2000 1 m^3 cages of 20 mesh per inch Saran screening were set up on the plots.

On June 8 an additional field-collected bolting plant was transplanted into the centre of each plot, as the transplanted rosettes were very slow to bolt in comparison to field-grown overwintered plants. Insects were placed in treatment cages as follows:

10 *M. edentulus* (sex not determined) June 8 – June 16
20 *O. hookeri* (sex not determined) June 1
150 female, 100 male *R. tripleurospermi* May 16 – June 21

Plots were monitored weekly from July 5, until August 23, 2000 and the number of open flowers and ripe seed heads in each plot was recorded, with ripe seed heads being collected. The experiment was harvested between August 28 and September 13. All plants were cut at soil level. For two of the five plants in each plot (plants 1 and 3), the number of stems was counted, and the height of each stem was measured. Plant 1 was the plant in the north-west corner of the plot while plant 3 was the additional bolting plant transplanted into the centre of the plot on June 8. Because of the large size of many of the plants it was not feasible to take stem counts and height on all plants. Total above-ground biomass, number of flowers and ripe seed heads, and seed weight and number were determined for all plants. Numbers of *M. edentulus* exit holes, *R. tripleurospermi* galls and adult *O. hookeri* emerging from the harvested seed heads were recorded.

3.3.2. Factorial plot experiment 2001

As the 3-insect impact experiment did not give satisfactory results in 2000, it was repeated in 2001 on a new site at ARC Vegreville. The layout and design were the same, except that a single scentless chamomile plant was transplanted into the centre of each plot. Plants used in 2001 had been seeded in 10-cm square pots in the greenhouse in July 12, 2000 and had grown to the rosette stage by August 29, 2000 when they were placed outside to overwinter. Plants were transplanted into the plots on April 25, 2001 and watered frequently until established, as field conditions were very dry in spring 2001. The cage screens were put in place on May 25, 2000. Insects were introduced into the appropriate cages as follows:

5 ♀ 2 ♂ *M. edentulus* May 29 – June 5
70 *O. hookeri* (sex not determined) June 13 – July 6
40 ♀ 4 ♂ *R. tripleurospermi* June 4 – July 5

Plots were monitored weekly from July 4 to August 22 and the numbers of open flowers and ripe seed heads counted, ripe seed heads being collected.

The experiment was harvested from Sept. 6 to Sept. 17. All plants were cut at ground level, the height of each stem was measured and the numbers of *M. edentulus* exit holes, *R. tripleurospermi* galls, open flowers, and mature seed heads recorded. Stems, seed heads and flowers were dried and weighed, seed was separated, cleaned,

weighed, and seed number estimated by weighing and counting a subsample using an automatic seed counter (Seedburo Count-a-Pak 801-10/B). Adult *O. hookeri* emerging from the harvested seed heads were collected and counted.

3.3.3. *Rhopalomyia tripleurospermi* alone: field population study 2001-2002

On November 16 2001, 50 galled and 50 ungalled scentless chamomile rosettes were selected from a field population on a creek bank on the east side of the ARC Vegreville site and marked with wire pin flags pushed into the soil on the north side of each rosette as close as possible to it. Galled plants were marked with orange flags and ungalled ones with blue flags. Conditions were very dry and most rosettes were small. On July 12 2002, when the surviving overwintered plants were in flower, the site was revisited, surviving plants were counted, dug out, soil cleaned from the roots, and bagged. For each plant the number and height of stems, and the number of open flowers and flower buds were recorded. Plant material was dried at 70°C and aboveground biomass and root biomass determined separately. For the galled plants the remaining overwintered gall tissue at the base of the plant, which was dry and brown by mid-July, was also weighed separately.

3.3.4. *Rhopalomyia tripleurospermi* alone: transplant study 2001-2002

In a separate experiment, 30 rosette stage scentless chamomile plants in 10-cm square plastic pots were exposed to oviposition by *R. tripleurospermi* in the greenhouse between September 5 – 10 2001. An additional 30 plants in the same growth stage were kept as controls. Once gall development was apparent, all plants were placed outdoors in holding trays in a field cage for overwintering on October 1, 2001. Galled and ungalled plants were labelled with colour-coded tags and interspersed with each other in the holding trays. Plants were covered with Microfoam insulating plastic sheeting for protection. On May 9 2002, all 60 plants were transplanted to the field on the ARC Vegreville site in a fully randomized arrangement, in a 6 × 10 array with approximately 1 m spacing between plants. Plants were watered as necessary until they became established. By early July 2002, fresh *R. tripleurospermi* galls were beginning to appear on many plants in both the control and treated groups. All plants were dug up on July 4 2002, and counts and measurements were taken as in the previous experiment.

3.4. Overwinter survival of *Omphalapion hookeri*

On November 14 2001 four 1 m^3 cages (20 mesh per inch Saran screening) were placed on a mowed grass area behind the ARC Vegreville laboratory buildings, and four further cages were placed in an area of rough grass between field plots on the east side of the ARC site. On November 15, 100 *O. hookeri* adults were placed into each of three cages at each location. The adults had been obtained from an outdoor rearing cage and from the impact experiment, had been allowed to feed on caged scentless chamomile plants in the greenhouse for approximately one month, and had then been stored at 5°C prior to use. Adults were tipped out of a vial into the centre of the cage plot. Weather conditions at the time were overcast with a temperature of about 8°C. On November 21

the cage covers were removed to allow snow to accumulate on the plots. The covers were replaced on April 12 2002 and three potted scentless chamomile plants were placed into each cage to attract any emerging *O. hookeri* adults. The plants were watered and replaced as necessary and checked at 2 – 3 day intervals from April 22 to June 20. Any *O. hookeri* found on the plants were removed and counted.

3.5. Dispersal of *Omphalapion hookeri* and *Rhopalomyia tripleurospermi*

In July and August each year from 1999 to 2002 the dispersal of *O. hookeri* and *R. tripleurospermi* from the Vegreville release sites was mapped. The area around the release sites was searched by vehicle in a grid pattern along the township and range roads. Representative patches of scentless chamomile were inspected and the presence or absence of *O. hookeri* and *R. tripleurospermi* galls was noted. The location of each plant checked was mapped using a portable GPS unit (Corvallis March II™). The search was continued outwards in an expanding pattern until no further *O. hookeri* or *R. tripleurospermi* were found. GPS data were transferred to a geographic information system (MapInfo 4.0) and maps were generated to show the area of dispersal for each insect and year.

3.6. *R. tripleurospermi* plot samples and phenology data

The phenology of *R. tripleurospermi* populations in the field was studied by several different sampling methods.

3.6.1. Stem sampling from the field

On June 30 2000 a 10 m × 10 m area was marked out in an area infested with scentless chamomile on the east side of the ARC Vegreville site. This was immediately east of a low spot or creek running through the property where standing water is present in some years and is referred to as the "slough site". At weekly intervals a sample of 10 scentless chamomile stems was collected at random from this area, the number and location of galls on the stems was recorded, and any galls present were dissected to determine the number and stages of *R. tripleurospermi* present. This sampling protocol was repeated in 2001, except that a 20 × 20 m area was used and stems were collected from one or more randomly selected 0.1 m^2 quadrats within the area on each sampling date.

3.6.2. Emergence from gall samples

Samples of 20 galls were collected weekly from the field and brought into the laboratory, where they were kept at room temperature in covered petri dishes with moistened filter paper to maintain humidity. The dishes were checked daily and the numbers of emerged male and female adult *R. tripleurospermi* and parasitoids recorded up to 30 days after collection. In 2000 the samples were collected from July 24 to October 30 from the area immediately surrounding the original release plots on the ARC site. Collections in 2001 were made from May 3 to October 18. Initially galls were

collected from the same area as in 2000 but due to low gall populations sampling was switched on May 31 to the "slough site", about 200 m east of the release plots.

3.6.3. Weekly gall counts on release plots

Between May 9 and May 12 2000, all overwintered galls on the 6 release plots used in 1999 were tagged by placing a plastic tag in the soil directly north of the gall. The plots were inspected at weekly intervals and when new galls were found they were marked with a yarn tag attached to the stem on which they had developed (all new galls were on bolting stems). When new galls began to appear in the plots, on June 23 2000, six 0.1 m^2 sampling quadrats were set up in the plots for continuing sampling. At weekly intervals until November 2, all plants in these sampling quadrats were examined and any new galls counted and classified as axillary, terminal, rosette or flower galls. New galls were marked with yarn tags (in the case of galls on bolting stems) or plastic pot markers inserted into the soil (in the case of rosette galls).

On May 4 2001, six 0.1 m^2 sampling quadrats were marked out on the plots on which the initial releases of *R. tripleurospermi* had been made in 1999. All overwintered galls within the sampling quadrats were marked as in 2000. At weekly intervals until September 28, the quadrats were rechecked and the location and number of any newly appeared galls was marked and recorded as in 2000.

3.6.4. Emergence traps

To determine the timing of adult emergence from overwintered rosette galls, emergence traps were constructed from 16-dram clear plastic vials with the bottom removed and replaced with nylon mesh. Twenty traps were placed over overwintered rosette galls in the east field release plots on May 19-23 2000. The traps were checked daily from May 25 to June 10 2000 and all emerged *R. tripleurospermi* adults found in them were sexed and counted. In 2001 the traps were placed in position on May 7 and checked until June 7.

3.7. *Napomyza* sp. near *lateralis* biology and host-specificity screening

All studies on *Napomyza* sp. near *lateralis* were carried out at the CABI Bioscience Station at Delémont, Switzerland, by Dr. Hariet Hinz, with funding provided by ARC from ADF and AARI.

3.7.1. Field sampling on non-targets

On 27 April 1999, 28 *Matricaria recutita* plants were randomly sampled from a commercial chamomile field near Basle in Switzerland.

3.7.2. No-choice adult feeding and oviposition tests

Between 29 July and 10 December 1999, 146 no-choice starvation feeding and oviposition tests were set up with 16 critical test plant species with 5-12 replicates each. One newly emerged pair each of *Napomyza* sp. near *lateralis* was released into a Petri

dish (9 cm diameter) with a gauze top, and provided with cut leaves and/or buds of one test plant species or the control (*T. perforatum*), inserted into a moist horticultural sponge block. Each day that a series of test plants was set up, at least one control dish was set up as well. The bottom of the Petri dish was lined with filter paper, and a little piece of moistened tissue was added to provide additional humidity. The dishes were kept in an incubator set to constant 20°C and 16:8 hrs. (light: darkness). The plant material was exchanged every 48 hrs, and the number of feeding holes and eggs laid recorded until the flies died. Dead females were dissected, and the number of mature eggs present in the ovaries recorded.

3.7.3. No-choice development tests

Between 23 April and 16 August 1999, 181 no-choice tests (144 test plants and 37 controls) were set up, including a total of 49 test plant species with 1-4 replicates each. Two females and 1-2 males were transferred into transparent plastic cylinders or in Petri dishes and provided with cut scentless chamomile shoots or leaves. When females started to lay eggs, the flies were released onto single potted gauze-covered plants. Each day that a series of test plants was set up, at least one scentless chamomile control plant was set up as well. In addition, it was assured that test- and control plants were of similar size and in the same phenological stage. After 10-14 days, the plants were searched for surviving flies which were re-used for further tests, provided they were still laying eggs. All plants were put back into gauze-covered cages to protect them from further attack by naturally occurring *Napomyza* spp. Six to eight weeks after infestation the plants were dissected for signs of attack.

3.7.4. Single-choice development tests

In addition, 18 single-choice tests were set up between 30 June and 27 August 1999 with six critical test plant species, i.e., on which development had occurred previously in no-choice tests. One test and one control (scentless chamomile) plant were potted together, covered with a gauze bag, and exposed to 2-4 females and 1-4 males each for 12-17 days.

3.7.5. Multiple-choice field cage tests

On 9 May 1999, a multiple-choice field cage was set up with three plants each of six test plant species (*Chamaemelum nobile, Chrysanthemum carinatum, Chrysanthemum coronarium* 'Big Leaf', *Tanacetum parthenium, Lonas annua,* and *Bidens cernuus*), and three plants each of two *T. perforatum* biotypes. Between 9 and 18 May, a total of 70 females and 60 males were released into the cage. All plants were left in the cage and dissected 6 – 8 weeks later for signs of attack.

3.7.6. Open-Field Tests

On 27 April 2000, an open-field test was established for *Napomyza* sp. near *lateralis* with five critical test plant species: *Matricaria recutita, Anthemis cotula, A. tinctoria, Chrysanthemum carinatum,* and *Tanacetum parthenium*. *Chrysanthemum coronarium* 'Big

Leaf' was not used in this test, because plants are not frost hardy. Five plots were established, one central plot and four satellite plots (Figure 2). To reduce attack of plants by *Napomyza* occurring naturally in the Centre's garden, the plots were established about 80m away from the garden beds, where test and control plants are grown. In each plot, four potted plants of each of the five test species and the control, *Tripleurospermum perforatum*, were arranged in a randomized block design. On 29 April, the number of shoots, maximum shoot height or length of longest leaf and the phenostage were recorded for all plants. An additional 30 scentless chamomile plants that were attacked by *N.* sp. near *lateralis* were placed in the central plot as a source of infestation. The leaves of these source plants were cut back to reduce the possibility of feeding and oviposition on them. Twenty-seven similarly attacked plants of scentless chamomile were placed in cages and fly emergence checked daily to obtain an estimate of the number of flies emerging from the plants placed in the central plot. Between 28 April and 7 May, all plants were checked six times for *Napomyza* adults to see on which plants they preferentially land. On 20 June, the scentless chamomile control plants in the central plot, and one control plant in each satellite plot were dissected for agromyzid attack. On 23 June, all control plants dissected as well as senescing test plant species were replaced by new plants of the same species, i.e. all *Matricaria recutita* plants, and three *C. carinatum* plants each in the central and the two upper satellite plots. Because attack of scentless chamomile plants dissected was low, 148 females and 97 males of *Napomyza* sp. near *lateralis* were released onto the central plot between 29 June and 30 July. Between 21 July and 11 September, all test and control plants exposed in the central plot and the four satellite plots were dissected. Unfortunately, the plants that had been placed in the plots as *Matricaria recutita* were in fact scentless chamomile plants, but this only became obvious when the plants flowered The number of dead, parasitized and alive larvae, pupae, and mines were recorded separately for each plant. All pupal cases found were incubated in ELISA-dishes for adult emergence.

On 5 May 2001, a second open-field test was established at Delémont with the herbal chamomile, *Matricaria recutita*, and scentless chamomile. Sixteen rosettes and ten bolting plants of each species were arranged randomly in a garden bed and left exposed for infestation by *Napomyza* occurring naturally in the Centre's garden. On 20 and 21 June, five plants of each species were dissected. Two more plants of each species were dissected for attack at the beginning and at the end of July.

3.7.7. Continuation Trial

A continuation trial was established in spring 2000 to test whether critical non-target species can support successive generations of *N.* sp. near *lateralis* under no-choice conditions. Between 21 April and 22 May 2000, nine no-choice cages were established, six with critical test plant species (*Anthemis sancti-johannis, A. tinctoria, Chrysanthemum carinatum, C. coronarium* 'Big Leaf', *Tanacetum parthenium*, and *Helianthus* ssp. *lenticularis*), and three cages with scentless chamomile control plants. Two to five individually potted plants of one test species or the control were placed in each screen

cage (40 x 40 x 75 cm or 30 x 30 x 55 cm), and between 10-20 pairs of *Napomyza* released into each. The cages were kept outside underneath a shelter. After about two weeks (12-15 days), the cages were checked for surviving flies, and these reused. Afterwards the plants were kept in a larger gauze-covered field cage (2 x 2 m) to protect them against *Napomyza* and parasitoids occurring naturally in the Centre's garden. Five weeks after infestation the plants were replaced into the smaller gauze cages for adult emergence, each plant species in a separate cage. Emerging adults were collected every second day, their number and sex recorded, and transferred onto new plants of the same plant species they emerged from. Using this method, nine cages (two with control plants, and seven with test species) were established with the second generation of *Napomyza* emerging, and 16 with the third generation (eight with control plants and eight with test species). One to eight individually potted plants of one test species or the control were exposed in each cage.

Each cage was treated as one replicate. The number of offspring produced by each female was calculated by dividing the number of flies that emerged in a cage by the number of female flies that had originally been released. Development time was calculated as the number of days from release until 50% of flies had emerged. When the test was terminated on 16 November, not many flies had emerged from the *C. carinatum* plants, so these plants were dissected to record *Napomyza* larvae and pupae. Scentless chamomile plants of two cages, established with the first generation of *Napomyza*, were dissected prior to adult emergence to obtain pupae for cross-breeding experiments. They were therefore not included in the results.

3.7.8. Taxonomy: Mitochondrial DNA studies

In 1999, additional *Napomyza* sp. near *lateralis* specimens originating from the rearing colony at CBI Bioscience, as well as from scentless chamomile plants collected in Eastern Austria, were transferred into 100% alcohol and sent together with *Napomyza* specimens from Russian knapweed to Dr. Sonja Scheffer (USDA, Beltsville). All larvae found on test plants and adults emerged in the 2000 open-field experiment were transferred into 100% alcohol and sent to Dr. Scheffer for DNA analysis, to confirm that they originated from *T. perforatum N.* sp. near *lateralis* biotype. On 19 October 2000, an additional consignment of *Napomyza* specimens from different host plants and geographical regions was sent to Dr. Scheffer to carry out further analyses. The shipment contained: *Napomyza* sp. near *lateralis* adults and larvae originating from *Acroptilon repens* from four sites in Turkey and one in Uzbekistan, adults originating from *T. perforatum* from one site each in Switzerland, England and Turkey (only one adult), and adults of *Napomyza cichorii* reared from *Lactuca sativa* L.

3.7.9. Taxonomy: Cross-breeding experiments

Two experiments were conducted. Newly emerged females from *T. perforatum* were mated with newly emerged males from *A. repens*, and newly emerged females from *A. repens* with males from *T. perforatum*.

1) Individual pairs were transferred into Petri dishes (15 cm diameter) and provided with cut leaves of the host plant the female originated from. The leaves were inserted in a moist block of horticultural foam to keep the material fresh, and the dish lined with filter paper to avoid condensation. The leaves were changed every two days, and carefully dissected for eggs under a stereo-binocular microscope. Eggs were incubated in a Petri dish (5 cm diameter) lined with moist filter paper. The dishes were checked daily for hatching larvae and these removed. This was carried out during the whole life span of females.
2) Individual pairs were transferred onto a potted gauze-covered plant of *T. perforatum* or *A. repens*. After 2-4 days the plants were searched for surviving flies and these reused.
As far as possible, pairs originating from the same host plant were established as controls in both experiments.

4. RESULTS AND DISCUSSION

4.1. Rearing, release, and monitoring of *Microplontus edentulus*

The rearing success with *M. edentulus* was variable but larval emergence was generally low (Table 3). In contrast, in 1997 an estimated 3,350 larvae emerged as the progeny of 142 adults from the first shipment of *M. edentulus*. The reasons for the low reproductive success in field cage rearing between 2000 and 2002 are not known. In 2000, suitable bolting plants were not available for oviposition when the shipment of *M. edentulus* was received from Europe, and the adults were held in vials at 10°C with chamomile foliage for 13 days until they could be transferred to plants. This may have imposed a stress on them that adversely affected their fecundity. However, the 2001 shipment was received later in the season and immediately transferred to suitable plants in a field cage, but also had low reproductive success.

Observation in the rearing cages showed that *M. edentulus* larvae frequently tunnelled up from the oviposition site through the branches to the base of a flower head and completed most of their development there, emerging when mature from an exit hole in the base of the receptacle. Larvae feed in the receptacle tissue without damaging the developing seeds, so this has minimal impact on scentless chamomile reproduction. This mode of development had not been reported in the biological studies conducted in Europe prior to introduction of *M. edentulus* (Hinz et al. 1996).

Microplontus edentulus is definitely established at two sites, Vegreville, Alberta, and Nipawin, Saskatchewan (Table 1). In addition it may be established at Edmonton, Alberta, and Lang, Saskatchewan. At these sites evidence of breeding was seen in the year of release but not in subsequent years. There is some suggestion in the data that caged releases are more likely to result in successful establishment: three out of five caged releases of adults resulted in confirmed or possible establishment, while none of eight open releases of adults did so. An alternative explanation may be that caging makes establishment easier to detect by preventing adult dispersal immediately after release and forcing the initially released adults to breed at the release site.

4.2. Rearing, release, and monitoring of *Rhopalomyia tripleurospermi*

4.2.1. Rearing

Greenhouse rearing of *R. tripleurospermi* was generally easy and rapid, and the limiting factor was usually the space required to produce sufficient fresh rosette-stage scentless chamomile plants for infestation. Under greenhouse conditions, gall development became visible as early as 7 days after exposure to ovipositing females but usually after around 13 days, and adult emergence began as early as 24 days but usually around 35 days after exposure to oviposition (Figure 3).

Two natural enemies were found in *R. tripleurospermi* rearing colonies that had a significant impact on production. A parasitoid wasp, *Mesopolobus* sp. (Hymenoptera:

Pteromalidae), was found in the greenhouse rearing cages in late 1999 and from August 2000 onwards. Galled plants that were stored over the 2000-2001 winter produced mainly adult parasitoids and only few *R. tripleurospermi*. The colony was cleaned up by discarding cages in which parasitoids were emerging and rearing from fresh galled plants collected from the field, ensuring that no parasitoids were transferred into the oviposition cages.

In July 2001 it was also found that western flower thrips (*Frankliniella occidentalis* Pergande) were feeding on eggs of *R. tripleurospermi* in the greenhouse colony, severely reducing its viability. Plants exposed to oviposition in the greenhouse between May 29 and July 9 had produced very few galls by mid July. Examination of shoot tips recently exposed to oviposition showed many shrivelled and collapsed eggs of *R. tripleurospermi*. The plants were found to have high populations of adult and larval western flower thrips, many of which had an unusual reddish-orange colouration unlike the usual creamy white colour of larval WFT. This suggested that the thrips had been feeding on *R. tripleurospermi* eggs, which have a strong red pigmentation. A feeding trial was set up to test this hypothesis. A single scentless chamomile leaf piece with approximately 15 1-day old *R. tripleurospermi* eggs was placed in a 4.5 cm diameter petri dish along with approximately 20 WFT larvae collected from flowers of yellow toadflax (*Linaria vulgaris* Miller) in the same greenhouse. These thrips had not been exposed to *R. tripleurospermi* and had the typical creamy white colouration. Within 3 hr several thrips larvae were seen to have red-orange gut contents, and some *R. tripleurospermi* eggs were collapsed. One thrips was directly observed piercing a *R. tripleurospermi* egg and sucking its contents out within a few minutes. Within 24 hr all the eggs in the dish had been consumed.

In subsequent rearing, plants were monitored closely for thrips and predatory mites [*Hypoaspis miles* (Berlese)] were released as biological control agents for thrips.

4.2.2. Releases and monitoring

Overwinter field survival of *R. tripleurospermi* from the 1999 releases on the ARC Vegreville site was confirmed in early May 2000. A rosette gall of approximately 2 cm diameter was collected from the plot area on May 5, 2000 and was found to contain live pupae when dissected. The gall was left in a petri dish in the laboratory and between May 8 and May 16, 44 adult *R. tripleurospermi* (28 ♀ and 16 ♂) had emerged from it. A further 10 galls were collected on May 9 and from these a total of 127 ♀ and 55 ♂ emerged. Rosette galls on the six release plots were counted and tagged on May 9 – 12, 2000. A total of 519 galls were found, equivalent to 12.9 galls m^{-2}. On May 25 all galls were collected from one half of each of the plots. A total of 179 galls were collected and held in jars in the laboratory for emergence. Total emergence from these galls was 385 ♀ and 210 ♂. These adults were used in the impact experiment and for greenhouse rearing.

On May 19 2000, four healthy galls were collected from the release site near Bruce, Alberta (AB-99-0004). These were held in vials in the laboratory for emergence and produced a total of 8 ♀ and 4 ♂ adult *R. tripleurospermi*.

The most intensively monitored releases of *R. tripleurospermi* were the 47 releases made in Alberta in 1999 (Table 2). In addition to these, material was provided for 125 additional releases in 1999 - 2002 in Alberta and other provinces. These are summarized in

Table 4. Most of the releases in Alberta in 2001 and 2002 were made by users (landowners, consultants, and municipalities) using infested plants provided from ARC's greenhouse rearing on a fee-for-service basis. The 14 releases in Saskatchewan in 2002 were redistributions made by transplanting galled plants from previous release sites into new locations (G. Bowes, pers. comm.).

Overall, 74% of releases of *R. tripleurospermi* in Alberta in 1999 resulted in successful establishment (

Table 5), defined as galls found near the release site one year or more after release. Populations of *R. tripleurospermi* have persisted for up to 3 years after release at several sites. Releases made using caged adults apparently have a slightly higher success rate than those made by transplanting infested plants (

Table 5) However, this is probably a result of most adult releases having been made earlier in the season. Although successful establishment was achieved from releases made throughout the growing season, percent success declined from 92% for releases made up to July 15 to 60% for releases made from mid-August to early September (Table 6).

4.3. Impact evaluation

4.3.1. Factorial plot experiment 2000

Growth and development of the scentless chamomile transplanted into plots in the 2000 factorial experiment was not typical of that normally observed in the field. Greenhouse-grown rosette stage plants were transplanted on May 1 and required frequent watering due to dry conditions in the field. By early June these plants had developed into large vigorous rosettes, but there was no sign that they had begun to bolt, as would normally be occurring in the field by this time. At this time it was decided to transplant an additional bolting plant into each cage to ensure that stems and flower buds would be available for attack by *M. edentulus* and *O. hookeri* respectively. The original transplanted rosettes began to bolt at the end of June, by which time these rosettes had developed into very large, lush plants with multiple growing points. Each of these plants eventually produced a mean of 25 flowering stems per plant.

It is possible that the greenhouse-grown plants used for the original transplants were not exposed to the correct temperature and photoperiod conditions to trigger bolting and flowering at the normal time of year.

By the time plants were in full bloom in mid August the 1 m^3 cages were completely filled with scentless chamomile. The large bulk of the plants made complete harvesting and data collection from all plants impracticable. In each cage only one of the original plants (plant 1, in the NW corner of the cage) and the additional transplant (plant 3, in the centre of the cage) were harvested.

The cages were not fully effective in excluding *O. hookeri* and *R. tripleurospermi* from the plants assigned to the treatments without these insects (

Table 7). Total emergence of *O. hookeri* was actually greater from both plants 1 and 3 in the cages assigned to treatments without this insect. *Rhopalomyia tripleurospermi* gall numbers were significantly higher only on plant 1 in cages assigned to *R. tripleurospermi* treatments. The cages were successful in confining *M. edentulus*: both plants 1 and 3 showed significantly more exit holes in cages assigned to treatments with *M. edentulus*.

Treatment effects on plant performance parameters were therefore estimated only for *R. tripleurospermi* on plant 1 and *M. edentulus* on both plants 1 and 3. No significant effects were found (Table 8).

4.3.2. Factorial plot experiment 2001

Plants used in the 2001 experiment had been seeded in the greenhouse in fall 2000, grown to the rosette stage, and then overwintered outdoors before transplanting to the field in spring 2001. They had therefore been exposed to the same temperature and photoperiod conditions as field-grown plants, and bolted and flowered at the appropriate time. Although only a single plant was transplanted to each cage, these plants also grew very large and vigorous, filling the 1 m^3 cages by the time the experiment was harvested.

In 2001 the cage treatments were largely successful in confining insects to the appropriate treatment cages (

Table 9). Although some insect attack by all species was found in control cages, all "with" cages had significantly larger numbers of the treatment insect than the corresponding "without" cages. The plants again grew extremely large and vigorous, each plant virtually filling a 1 m^3 cage by the time the experiment was harvested.

Treatment effects in the 2001 experiment were estimated by 4-way analysis of variance for *O. hookeri*, *M. edentulus*, and *R. tripleurospermi* (presence or absence of each) and blocks (Table 10). Significant effects were observed for *R. tripleurospermi* on mean stem height and number of flowers, for *O. hookeri* on number of flowers, number of seed heads, and total seed weight, and for *M. edentulus* on number of seed heads. (Seed heads were counted as those judged to contain ripe seed at the time of harvest, while flowers were heads which had not yet matured seed.) Estimated least-square means for significant treatment effects are shown in

Table 11. *R. tripleurospermi* had a small but significant effect in reducing mean stem height and number of flowers. This is to be expected from the type of damage caused by *R. tripleurospermi* as galling on terminal buds tends to stunt the growth of main stems and delay flower production. The reduction in seed weight due to *O. hookeri* is also not unexpected, as *O. hookeri* directly reduces seed production by larval feeding. The effects of *O. hookeri* and *M. edentulus* on flower and seed head numbers are less easy to understand. *O. hookeri* reduced the number of flowers and increased the number of ripe seed heads, with no overall significant effect on the number of heads. *M.*

edentulus also significantly increased the number of ripe seed heads. Possibly feeding by these insects increases stress on the plant, leading to more rapid maturation of flower heads.

Overall there were no major impacts of the insects on performance of scentless chamomile plants in the 2001 experiment. In the absence of competition, the plants grew even larger and more vigorous than those in the 2000 experiment, and much larger than those typically seen in the field. The lack of significant impact in these experiments does not necessarily indicate that the insects would have no effect on scentless chamomile plants of more normal size growing under field conditions. This suggests that future evaluation of the biocontrol agents for scentless chamomile should focus on their effects on the competitive ability of scentless chamomile growing intermingled with a crop or other vegetation.

4.3.3. *Rhopalomyia tripleurospermi* alone: field population study 2001-2002

Because of dry conditions in the field in the fall of 2001 and spring of 2002, plants in the field population were very small and stunted and there was considerable overwinter mortality in both the galled and ungalled groups. From 50 plants in each group, 24 galled plants and 29 ungalled plants were found to have survived to July 12 2002. Survival was slightly, but not significantly, lower in the galled group (48% versus 58%, $\chi^2 = 0.3164$, 1 d.f., $p=0.3164$). Galling appeared to increase the number of stems produced by surviving overwintered rosettes in the following summer. All ungalled plants produced a single flowering stem, while galled plants produced a range of 1 - 4 flowering stems (mean 1.83). This suggests that galling may disrupt apical dominance, allowing more buds to develop into bolting stems after overwintering. Total biomass and number of flowers were slightly, but not significantly increased in the galled group (Table 12).

4.3.4. *Rhopalomyia tripleurospermi* alone: transplant study 2001-2002

Plants used in the transplant study were larger and more vigorous than those available for the field plot population. There was no mortality in either the galled or control groups during outdoor overwintering in pots. On harvesting the experiment it was found that one transplant in the control group had actually consisted of two rosettes in one pot. Data from these plants were discarded in the analysis, reducing the sample size for the control group to 29. As seen in the plants from the field population, *R. tripleurospermi* had significant effects on plant architecture. Overwintered galled rosettes produced 97% more bolting stems in the following season than controlled plants, but these stems were on average 26% shorter than stems developing on ungalled plants (

Table 13). Total biomass and flower numbers were slightly but not significantly reduced on galled compared with ungalled plants.

4.3.5. Overwinter survival of *Omphalapion hookeri*

Adult *O. hookeri* began to be found on plants in the cages on May 27 2002. Unfortunately, more adults were found per cage in the control cages, where *O. hookeri* had not been released in the fall of 2001, than in cages where 100 *O. hookeri* had been released (Figure 4). This suggests that either there was a large background population of *O. hookeri* already present in fall 2001, or, more likely, that *O. hookeri* from surrounding areas were able to enter the cages from outside in spring 2002, attracted by the scentless chamomile trap plants. It was thus not possible to estimate the overwinter survival rate of *O. hookeri* from 2001 to 2002.

4.3.6. Dispersal of *Omphalapion hookeri* and *Rhopalomyia tripleurospermi*

The dispersal of the two agents from the release sites at Vegreville in 1993 and 1999 respectively is shown in Table 14 and Figures 5 – 8.

Omphalapion hookeri initially dispersed fairly slowly, and up to 3 years after release was found only 180 m from the release plots. Its spread then began to accelerate (Figure 5). From 1996 to 2002 it dispersed at a fairly uniform rate with a mean velocity of 2.8 km yr^{-1} (Figure 6). The furthest spread found was 15.6 km from the release site in 2002. The area over which the weevils were found declined somewhat in 2002 in comparison with the previous year. This is probably due to low population densities in that year, reducing the chances of finding weevils at the periphery of their range. In 2002, a severe early spring and summer drought reduced the number of scentless chamomile plants flowering during the normal oviposition period of *O. hookeri*, reducing the numbers of adults emerging later in the summer when mapping was conducted.

Initial dispersal of *R. tripleurospermi* was much more rapid than that of *O. hookeri* (Figure 7). From 1999 to 2002 it spread at a mean rate of about 5.2 km yr^{-1}(Figure 8), and by 2002 it had already caught up with *O. hookeri* at 15.6 km from the release site. The rapid dispersal of *R. tripleurospermi* is somewhat surprising considering the fragility, small size, and short lifespan of the adults. It is probably due to the more rapid generation time of *R. tripleurospermi* (three generations per year as opposed to one for *O. hookeri*) which allows more rapid population increase and more frequent opportunities for dispersal.

4.4. *R. tripleurospermi* plot samples and phenology data

4.4.1. Stem sampling from the field

Numbers of galls and stages of *R. tripleurospermi* found in weekly samples of 20 stems from the slough site in 2000 are shown in Figures 9 and 10. Numbers stayed fairly low until mid August when they increased rapidly to around 5 galls per stem. This probably coincides with the appearance of mature galls of the F1 generation. Most samples in 2000 contained a mixture of all developmental stages of *R. tripleurospermi*, although late season collections from mid October onwards contained primarily larvae, suggesting that this is the main overwintering stage.

The gall counts from 2001 (Figure 11) show a clearer pattern of three distinct peaks in abundance in early July, mid-August, and mid-September, reflecting the three generations shown in emergence from field-collected gall samples. Pupae begin to appear after July 3 (Figure 12), indicating that the F1 generation reaches maturity about that time. Samples later in the season show a mixture of developmental stages of *R. tripleurospermi*, probably reflecting increasing overlap between generations due to individual variability in developmental rates. The gall count data for 2001 also show clear seasonal shifts in the plant phenological stages attacked. Rosette galls are present in the spring (surviving from the previous year), and from late August onwards. In summer all galls develop on terminal, axillary, or flower buds on bolted scentless chamomile plants.

The 2001 field sample data probably give a clearer indication of the phenology of *R. tripleurospermi* than the 2000 data due to a modification of the sampling procedure. In 2000 stems were sampled haphazardly from the whole 20 × 20 m sampling area, probably resulting in a bias towards stems with visible galls and an undersampling of small and rosette-stage plants. In 2001 a true random sampling procedure was used with a randomly placed 0.1 m^2 quadrat, and all stems within this were sampled until a total of 20 had been reached.

4.4.2. Emergence from gall samples

Adult *R. tripleurospermi* emerged from field-collected gall samples held in Petri dishes in the laboratory up to 30 days after collection. Numbers of adults emerging in 2000 and 2001 were plotted against collection date and time since collection (Figures 13 - 14). These plots allow an estimation of the developmental stage of larvae in the galls collected, as well as absolute numbers developing per gall. The collection date is shown along the front axis, and the time elapsed from gall collection to adult emergence is shown along the left-hand side axis. Thus midges which were almost mature (pupae or late larvae) when collected, and therefore emerged a few days later, are plotted near the front of the graph. Midges which were in an early larval stage when collected, and therefore emerged up to 30 days later, are plotted near the back.

As the results for the 2001 season (Figure 14) are clearer and more complete, they will be discussed first. Galls collected up to late May all completed emergence within

about 8-9 days of collection. These must have contained overwintered late larvae or pupae. There is a very clear first generation (F1) appearing in late May to early June and becoming mature by early July. These have completed emergence by mid-July. As adults live only a day, the next generation (F2) must therefore have been initiated in early to mid July. Although the numbers are not very high, the data suggest that the F2 generation begins to show up in the samples in late July, and is mature by late August to mid September. There is a much higher peak (bottom right of the graph) that begins to show up as numerous early stage larvae in early to mid September. These could hardly be the offspring of adults that emerging from F1 galls and oviposited in early July, around 9 weeks previously. Thus this appears to be the F3 generation. They are mature and emerging in large numbers by early to mid October. (In the field, most of these would presumably not emerge in the fall because of falling temperatures, and would form the overwintering generation. In our study they completed development and emerged because they were brought in to warm temperatures in the laboratory.) A few early stage larvae were collected in mid October (top right hand corner of the graph). These might be the beginnings of an F4 generation, or might just be late stragglers from the F3. Presumably they are unlikely to survive the winter.

The relative sizes of the peaks for the three generations probably reflect the availability and suitability for gall development of different growth stages of scentless chamomile throughout the growing season. The F1 galls develop on vigorous vegetative or early bolting stage scentless chamomile plants in early summer. These plants probably provide abundant resources for gall development and tend to produce large rosette or terminal galls. Terminal galls on early-stage bolting stems tend to stop the elongation of the stems and produce a "drumstick" appearance. In contrast, the F2 generation generally develops in late July to early August on plants that have completed most of their vegetative growth and are putting resources into flower and seed production. Available galling sites on these plants are primarily axillary or flower buds that provide only limited resources for gall development. On a per-gall basis, as the data were collected, this generation is therefore much smaller. The F3 generation develops on overwintering rosettes which are again more suitable for development of large multichambered galls. This seasonal pattern would likely be modified from year to year depending on precipitation patterns and the resulting seasonal abundance of different growth stages of scentless chamomile.

Data collection began later in 2000 and the patterns are not as clear (Figure 13). However, by overlaying the graph on the 2001 graph, it can be suggested that the 11 adults that emerged within 4 days from samples collected on July 24 and July 31 represent the very tail end of the F1 generation. This would suggest that the F1 generation was slightly later in 2000 than in 2001. Most of the adults that emerged within 15 days from samples collected after August 1 are the F2 generation, and the large peak at the bottom right of the graph, after October 1, represents the F3

generation. The clusters of early stage larvae in October (top right) are probably stragglers from the F3.

As development of *R. tripleurospermi*, like that of any insect, is temperature-dependent, the data for emergence in 2001 were also plotted on a degree-day scale (Figure 15). A degree-day model calculated from data from Hinz (1998) indicated that *R. tripleurospermi* requires 477 degree-days above a threshold of 7.7°C to complete development. Accumulated degree-days from January 1, 2001, above this threshold were calculated from hourly air temperature data collected at the ARC Vegreville weather station. A total of 1125 degree-days above this threshold were available in 2001. The degree-day plot (Figure 15) shows that the overwintering generation completes development by about 200 DD, the F1 by about 600 DD and the F2 by about 1000 DD. Thus there seems to be a generation about every 400 degree-days. The data suggest that *R. tripleurospermi* completes development more rapidly in the field than the 477 degree-days predicted by the model. Possibly temperatures in the gall are slightly higher than weather station air temperatures.

Degree-day accumulations above 7.7°C were also calculated for each year from 1994 to 2001 from ARC Vegreville weather station data (Figure 16). There is considerable variation, from about 800 DD in 1996 to about 1300 DD in 1998. It appears that some years (e.g. 1995, 1996) will allow two generations of *R. tripleurospermi* while others (e.g. 1997, 1998, 2001) will allow three.

4.4.3. Parasitism

Parasitism was first detected in late 1999 from galled scentless chamomile plants which had been placed outdoors in rearing cages and returned to the greenhouse to collect emerging adults.

The weekly gall samples collected from the field in 2000 and 2001 allowed an estimate of parasitism rates. No parasitoids emerged from field-collected gall samples from early 2000 (Vegreville May 5, May 9, Bruce May 19) , suggesting that rates of parasitism on the 1999 – 2000 overwintering population were very low. Emergence from the weekly samples collected between June 24 and October 30 was also generally low, with a slight peak in early August (Figure 17). Mean percentage parasitism (numbers of parasitoids emerged divided by the total *R. tripleurospermi* and parasitoids emerged) over the 2000 growing season was 12.2%. In 2001 mean parasitism increased to 32.0%, with three peaks of parasitoid emergence in late May, early July, and late September (Figure 18).

Samples from collected from 1999 to 2001 were sent for identification to Dr. Gary Gibson, Agriculture and Agri-Food Canada, Ottawa. At least two unidentified species of *Mesopolobus* (Hymenoptera: Pteromalidae) were present. Sorting of the specimens reared from field-collected gall samples in 2001 suggested that one of these emerged

from all three generations of *R. tripleurospermi*, while the second emerged primarily from the F3 generation. The genus *Mesopolobus* consists of several dozen species in North America, to which no key exists. No species of *Mesopolobus* has been recorded as a parasitoid of *Rhopalomyia* spp. in North America (pers. comm. G. Gibson 1999, 2002). These parasitoids must have originated from field populations on other hosts in Alberta, as none were observed during the initial rearing of *R. tripleurospermi* introduced from Europe in early 1999. The only parasitoid recorded from *R. tripleurospermi* in Europe was an *Aprostocetus* species (Hinz 1998).

4.4.4. Weekly gall counts on release plots

Gall counts in 2000 showed three clear peaks of gall development, in early July, mid August, and mid September (Figure 19), corresponding to the F1, F2, and F3 generations observed in emergence from weekly gall samples. A few new galls were observed in late October and early November. These developed on rosettes, and thus could potentially have survived the winter, but it is not known whether larvae in such early developmental stages can overwinter successfully.

Due to dry conditions on the plots in 2001, scentless chamomile plant density and gall numbers were much lower than in 2000 (Figure 20). Two peaks of gall appearance were observed, in mid June and mid August. These correspond to the F1 and F2 generations observed in emergence from the weekly gall samples, although the peaks observed in plot counts appear to have been observed slightly later than those in the emergence samples.

4.4.5. Emergence traps

In 2000, adult emergence in the traps placed over overwintered galls in the field plots began on May 25 and continued until June 2 (Figure 21). Male *R. tripleurospermi* were seen congregating around the outside of emergence traps containing live females, suggesting that they had emerged from galls elsewhere in the plots and were attracted by a female sex pheromone. Such pheromones are known to exist in several other gall midge species, including the pea midge, *Contarinia pisi*, the aphid predator *Aphidoletes aphidimyza*, the Douglas-fir cone gall midge, *Contarinia oregonensis*, and the orange wheat blossom midge, *Sitodiplosis mosellana* (Gries et al. 2000; Hillbur et al. 2001; Gries et al. 2002; van Lenteren et al. 2002).

Emergence in the traps was very low in 2001: only three females in total were found in the traps, between May 11 and May 14, suggesting that emergence began about two weeks earlier than in 2000. Although this is a small sample size, it is consistent with the degree-day model. In 2000, there was an accumulation of 124 degree-days above 7.7°C between January 1 and May 25. In 2001, 125 degree-days had accumulated by May 14. This suggests that emergence of overwintered *R. tripleurospermi* should be expected at an accumulation of about 125 degree-days.

4.5. *Napomyza* sp. near *lateralis* biology and host-specificity screening

4.5.1. Field sampling on non-targets

No attack of *Napomyza* was found upon dissection of plants from the commercial chamomile field near Basle.

4.5.2. No-choice adult feeding and oviposition tests

The longevity of *Napomyza* females did not depend on the plant species offered (1-way ANOVA: $F_{16, 129} = 1.60$, $P = 0.078$), and was on average 8.3 ± 0.39 days (range: 3-28 days) (Table 15). The mean number of feeding holes per female per day differed between plant species (1-way ANOVA: $F_{16, 127} = 9.09$, $P < 0.001$). A Duncan Multiple Range test showed that *Napomyza* females fed a similar amount on *Matricaria recutita, Anthemis sancti-johannis , Tanacetum vulgare, Chrysanthemum carinatum, C. coronarium* 'Big Leaf', *Coleostephus myconis*, and *Acroptilon repens* as on the control (scentless chamomile). Feeding on *Achillea millefolium, Dendranthema grandiflora* varieties, and *Helianthus annuus* ssp. *lenticularis* was particularly low, and not one single feeding hole was found on the *Cirsium arvense* leaves offered (Table 15).

The mean number of eggs laid also differed between plant species (1-way ANOVA: $F_{16, 127} = 6.90$, $P < 0.001$). A Duncan's Multiple Range test showed that significantly fewer eggs were laid on all test plants as compared to the control. On seven test plant species, no eggs were found at all: *Chamaemelum nobile, Achillea millefolium, Leucanthemum vulgare, D. grandiflora* varieties, *Lonas annua, H. annuus* ssp. *lenticularis*, and *C. arvense*.

In most plant species in which a choice between plant parts was offered, either more feeding holes and/or eggs were found in the leaves than in the stalks with flower buds, or no difference in feeding and oviposition was found between plant parts. However, on *D. grandiflora* feeding only occurred on the petals, and on *C. coronarium* feeding and oviposition was also observed on petals.

4.5.3. No-choice development tests

Of the 49 test plant species and varieties exposed, eleven supported the development of *Napomyza* sp. near *lateralis* (Table 16). In *Coleostephus myconis*, two empty pupae were found. Although all plants were protected in 1999, attack by *Napomyza* could thus not be 100% verified. In addition, a different, as yet unidentified fly species emerged from the pupae found in *C. myconis* in no-choice development tests in 1998 (Hinz and Kirkpatrick 1998).

A one-way ANOVA revealed that attack levels of the different cytotypes and plant origins of *T. perforatum* (,i.e. Canadian diploid and tetraploid, Austria and Rhine Valley) were not significantly different ($F_{3, 33} = 1.88$, $P = 0.152$), and they were combined. A comparison of attack levels of the pooled controls against the eight attacked test plant

species with more than two replicates revealed significant differences (1-way ANOVA after square-root transformation: $F_{8, 58} = 10.30$, $P < 0.001$). A Duncan multiple range test showed that only the closely related *Tripleurospermum ambiguum*, *Matricaria recutita*, and *M. discoidea* had similar attack levels as *T. perforatum*, while *Anthemis sancti-johannis*, *A. tinctoria*, *Tanacetum parthenium*, *T. vulgare*, and *Leucanthemum vulgare* were attacked to a lesser degree than the controls. Only one larva or pupa was found in *Lonas annua* and *Helianthus annuus* ssp. *lenticularis*, whereas 12 and eight individuals were found in the two *Chrysanthemum coronarium* 'Big Leaf' offered, respectively.

4.5.4. Single-choice development tests

One of the controls of *A. sancti-johannis* died, presumably due to a combination of adult and larval feeding of *Napomyza*, and attack by aphids. Of the six critical test plant species offered simultaneously with a control, three, *Chrysanthemum coronarium* 'Big Leaf', *Helianthus annuus* ssp. *lenticularis*, and *Bidens cernuus* were not attacked at all (Table 17). In *Lonas annua* and *Anthemis sancti-johannis*, only one of the three replicates (plants offered) was attacked, and the mature larvae found were dead. Only *M. recutita* was attacked to a higher degree, but still significantly less than the corresponding control (paired samples t-test: $t = 4.31$, d.f. = 4, $P = 0.013$). In the exceptionally strongly attacked controls (*T. perforatum*) of *Bidens cernuus*, many pupae were found in the flower heads.

4.5.5. Multiple-choice field cage tests

Of the six test plant species offered, only one replicate of one plant species, *C. carinatum*, was attacked by *Napomyza*, and only one pupa was found (Table 18). All other test plant species were unattacked, while all control (scentless chamomile) plants were infested by the agromyzid. In *T. perforatum* originating from Austria, three times as many larvae and pupae were found than in the Canadian tetraploid controls.

4.5.6. Open-Field Tests

Unfortunately, the plants that had been placed in the plots as *Matricaria recutita* were in fact scentless chamomile, but this only became obvious when the plants flowered, i.e. in July. Between 28 April and 15 May, 44 females and 51 males of *N.* sp. near *lateralis* emerged from the 27 scentless chamomile plants placed into cages to estimate the number of agromyzids emerging from the scentless chamomile source plants in the central plot. This was much less than expected. Not surprisingly, only one of the eight control plants dissected on 20 June had been attacked, and only one pupa was found. During final dissections, control plants that had been replaced were found to be attacked to a much higher degree (49.4 ± 10.3 (mean ± SE), $n = 8$) than control plants exposed in the plots from the beginning of the test onwards (7.4 ± 1.2, $n = 12$). Because the scentless chamomile plants used for replacement had been kept unprotected in the Centre's garden during May and June, they had presumably been attacked by naturally occurring *Napomyza*. To ensure comparability with test plants, it was therefore decided

to exclude the replaced control plants from the analysis, and instead include the scentless chamomile plants that had been thought to be *M. recutita* (see above). There was no difference in attack levels between plots (two-way ANOVA: $F_{4,85} = 0.592$, $P = 0.670$), while attack between plant species differed significantly ($F_{4,85} = 32.6$, $P < 0.001$). The control, *T. perforatum*, was preferred over all test plant species (Table 19). *Anthemis tinctoria*, *C. carinatum* and *T. parthenium* were found to be attacked to a very low degree. On *T. parthenium*, one mine was found in the receptacle of a flower head. The mine looked typical for *Napomyza*, but no larva, pupa or empty pupal case was found to confirm its origin. Whereas all scentless chamomile plants, and 70% of *A. cotula* plants were attacked, only 45% of *C. carinatum* plants, and about 30% of *A. tinctoria* plants were attacked, further emphasizing their lower acceptability (Table 19). In addition, *Napomyza* adults were only observed on *T. perforatum*, *M. recutita*, and *A. cotula*.

No signs of agromyzid attack were found in any of the plants from the second open-field test dissected during June and July 2001. Cool and wet spring conditions that year may have had an impact on the field populations of *Napomyza* sp. near *lateralis*.

4.5.7. Continuation trial

The continuation trial confirmed that *Napomyza sp.* near *lateralis* has four generations per year, only the first of which is distinct, while the following three generations are partially overlapping (Figure 22). The first generation emerged from 21 April to 6 May, the second from 15 June to 17 August, the third from 30 July to 29 September and the fourth from 8 September to 11 November.

As expected, the first generation of *Napomyza* developed on all plant species offered to some extent (Table 20). However, on *Helianthus annuus* ssp. *lenticularis*, only two pupae were found upon dissection, and from these only one *Napomyza* male emerged. Therefore, the test could not be continued. *Tanacetum parthenium* only supported one generation of *Napomyza* (Table 20). Development over two generations occurred on four of the six plant species tested: *Anthemis sancti-johannis*, *A. tinctoria*, *Chrysanthemum carinatum*, and *C. coronarium* 'Big Leaf'. In the two *Anthemis* species and *C. carinatum*, the number of *Napomyza* adults produced per female declined continuously over successive generations (Figure 23). No flies emerged in the fourth generation from *Anthemis* spp. Only *C. coronarium* 'Big Leaf' supported the development of the agromyzid over successive generations to a similar degree as scentless chamomile (Table 20, Figure 23). During the second generation, even more flies emerged over a shorter time period from *C. coronarium* than from *T. perforatum* (Table 20). However, only on *T. perforatum* did the total number of flies emerging increase exponentially (Figure 23).

4.5.8. Taxonomy: Mitochondrial DNA studies

Results of sequencing a 530 base-pair region of the mitochondrial cytochrome oxidase I (COI) gene indicate that individuals identified as *Napomyza* sp. near *lateralis* from

scentless chamomile and Russian knapweed respectively should probably be treated as two separate species (Figure 24; pers. comm. S. Scheffer, USDA, Beltsville).

4.5.9. Taxonomy: Cross-breeding experiments

Of eight crosses conducted with *T. perforatum* females and *A. repens* males, six laid eggs. Each female laid on average 4.7 ± 1.7 (SE) eggs, but no larvae hatched. In contrast, all seven *T. perforatum* females mated with *T. perforatum* males laid an average of 10.6 ± 2.6 eggs, but only 13.5% eclosed. This was a much lower percentage than anticipated, and presumably due to the fact that eggs of *Napomyza* are very sensitive to handling (and can easily be damaged). Only three of eight crosses established with *A. repens* females and *T. perforatum* males laid eggs. Out of the seven eggs laid, three larvae hatched (43%). On potted plants, development in cross-breeding pairs only occurred when *A. repens* females were mated with *T. perforatum* males, but not vice versa (Table 21). The results of both cross-breeding experiments conducted in 2000 confirm results obtained in 1999 (Hinz 1999b). Gene-flow is apparently only possible in one direction, i.e. when *A. repens* females are mated with *T. perforatum* males.

5. CONCLUSIONS

5.1. *Microplontus edentulus*

Low success in rearing the stem-mining weevil *M. edentulus* has limited the number of releases that could be made, and the amount of information that could be obtained on its effectiveness as a biological control agent for scentless chamomile. The insect is definitely established at one site each in Alberta and Saskatchewan, and evidence of breeding has been seen at one further site in each province. At Vegreville, Alberta, the field population has survived for five years from the original release. Transplantation of infested plants containing larvae of *M. edentulus*, before the mature larvae drop from the stems to the soil to pupate, appears to be the most successful release method, but is labour-intensive. If releases of adults are to be made, the results suggest that these should be caged rather than in the open.

No evidence was obtained from the field cage impact experiments in 2000 and 2001 of significant reduction in the performance of scentless chamomile plants due to attack by *M. edentulus*. In both years, the plants in these experiments grew much larger and more vigorous than typical field-grown scentless chamomile plants. It is possible that the impact of *M. edentulus* might be greater on plants of more typical size and vigour, or growing in competition with other vegetation. The tendency of many larvae of *M. edentulus* to tunnel up to the flower head and feed in the base of the receptacle, where they do not destroy any of the developing seeds, was previously unknown. This behaviour probably also reduces the impact of this insect on scentless chamomile.

One plant of scentless chamomile found at Vegreville in July 2001 showed severe wilting and browning of foliage on some stems. These stems were found to contain *M. edentulus* larvae. Unaffected stems on the same plant contained no larvae. Damage by the larvae may have provided an entry pathway for plant pathogens. Further studies should be made with *M. edentulus* to assess its relationship with fungal or bacterial plant pathogens, and the possibility of enhancing its effectiveness by using it as a vector for specific pathogens of scentless chamomile.

5.2. *Rhopalomyia tripleurospermi*

Successful and effective mass rearing and release methods have been developed for the gall midge *R. tripleurospermi*. The insect can be reared in the greenhouse on rosette stage scentless chamomile plants in 10-cm square plastic pots. Precautions must be taken to avoid the build-up of western flower thrips, which can have a severe impact on the colony by feeding on the newly-laid eggs, and of parasitoid wasps (*Mesopolobus* spp). Transplantation of 9 well-galled rosettes in early summer to a field site containing a healthy population of scentless chamomile normally results in successful establishment. *Rhopalomyia tripleurospermi* can also be established by releasing of adult midges into field cages placed over stands of scentless chamomile, but the difficulty of handling

adults and their lifespan of only a few hours makes this method less practical. The insect is now established at numerous sites across Alberta and Saskatchewan.

Field observations have shown that *R. tripleurospermi* normally completes three generations in a year in central Alberta, although this is temperature dependent. A generation is completed in about 400 degree-days above a threshold temperature of 7.7°C, slightly more rapidly than the 477 degree-days estimated from published rearing data (Hinz 1998). In some cooler years only two generations may be completed. Adult emergence in the field begins at about 125 accumulated degree-days since January 1.

Rhopalomyia tripleurospermi is attacked by at least two native parasitic wasps, *Mesopolobus* spp. (Hymenoptera: Pteromalidae). Average season-long rates of parasitism in the field at Vegreville, Alberta, increased from 12.2% in 2000 to 32.0% in 2001. To date this parasitism does not seem to have significantly impaired the ability of *R. tripleurospermi* populations to build up in the field.

Rhopalomyia tripleurospermi has shown excellent dispersal abilities in the field, despite the small size and short lifespan of the adults. At Vegreville, it spread out from the initial release site at an average rate of 5.2 km yr^{-1}, much more rapidly than the seed weevil *Omphalapion hookeri*.

Impact evaluation of *R. tripleurospermi* has shown some significant effects, although, as with the other insects studied, the exceptionally vigorous growth of scentless chamomile in the 2000 and 2001 test plots probably obscured the potential of the insect. Stem height was significantly reduced in plants attacked by *R. tripleurospermi* in both the 2001 cage experiment and the 2001 – 2002 overwintering rosette experiment. Reduction in plant height should impact the competitive ability of scentless chamomile growing surrounded by a crop or other vegetation. Flower numbers were also significantly reduced in the 2001 cage experiment.

Damage to overwintering rosettes of scentless chamomile by *R. tripleurospermi* has clear effects on plant architecture. Both in naturally infested plants in the field, and in the transplanted rosettes used in the 2001 – 2002 overwintering rosette experiment, galled rosettes produced significantly more flowering stems the following season. This was not accompanied by significant changes in total biomass or flower production. Thus it appears that overwintered galled plants produce more flowering stems in the following season, but that individual stems are shorter and produce less flowers than those produced by ungalled plants. The effects of this change in plant architecture on its competitive ability should be studied further. Although the data were not recorded, it is likely that stem elongation is also delayed by *R. tripleurospermi* attack in the first (F1) generation, as these galls frequently develop on the terminal buds of bolting stems, temporarily stopping further elongation. This should further decrease the competitive ability of scentless chamomile.

5.3. *Omphalapion hookeri*

The seed weevil *O. hookeri* is established at many sites across the prairies, and continued to disperse in the field around the Vegreville release site. Surveys from 1996 to 2002 showed that it is spreading out at a roughly constant rate of about 2.8 km yr^{-1}. Despite the large size of the test plants, *O. hookeri* produced significant reductions in scentless chamomile seed output in the 2001 field cage impact experiment. *Omphalapion hookeri* in the prairie provinces seems still to be free of attack by native parasitoids. As its numbers and range continue to increase in the field, it should make an increasing contribution to biological control of scentless chamomile.

5.4. *Napomyza* sp. near *lateralis*

Host range tests with *Napomyza* sp. near *lateralis* from scentless chamomile were completed. There appears to be no concern with attack on any related native North American non-target species. However, oviposition and development occur on a number of weedy, ornamental and minor economic species of European origin which occur in North America, including species of *Chamomilla*, *Chrysanthemum* and *Anthemis*. Attack rates on these in choice tests were always lower than on scentless chamomile. It should be noted that the *Chrysanthemum* species attacked do not include the common, perennial, florists' chrysanthemum, properly referred to as *Dendranthema grandiflora*, which is a major economic species. The herbal or German chamomile (*Chamomilla recutita*) is accepted as a host in cage tests, but there is a very low level of attack in the field. *Napomyza* sp. near *lateralis* is not considered a pest of commercial chamomile crops in Europe. German chamomile is not currently grown commercially to a significant extent in the US or Canada, but is grown in home gardens and is considered a potential economic crop.

The annual *Chrysanthemum coronarium*, grown for its edible leaves as an oriental vegetable, is accepted in no-choice tests. *Napomyza* sp. near *lateralis* could be reared through 4 generations on this host, increasing in numbers in each generation, although at a much lower rate than on scentless chamomile. The only commercial production area we have been able to identify for *C. coronarium* is in Riverside County, California, where a total of perhaps 15 acres are planted each year in late winter and disced under by April (pers. comm. M. Pitcairn, CDFA, 2001). It is not unlikely that *C. coronarium* may be grown in other areas with a large Oriental population and a strong market gardening industry, such as the Fraser Valley, British Columbia. However, Munro and Small (1997) state "In Canada *C. coronarium* grown as a vegetable is a curiosity of home gardens."

In an open-field test, stinking chamomile (*Anthemis cotula*), a weedy species, was consistently accepted, although the attack rate was less than 30% of that on scentless chamomile. *Anthemis cotula* is more widely distributed in North America than scentless chamomile, being reported from all 50 states (USDA - NRCS 2002). It could therefore

potentially act as a "bridging" host which would allow *Napomyza* sp. near *lateralis* to spread outside the distribution area of scentless chamomile.

Based on the information obtained in this study, the likelihood of significant non-target damage by release of *Napomyza* sp. near *lateralis* thus seems low. In particular, there seems to be no risk to any native North American plant species from this insect. However, there is uncertainty about the exact taxonomic status of *Napomyza* sp. near *lateralis*, there was damage to the commercial German chamomile (*Matricaria recutita*) in some tests, and there appears to be a possibility of attack on other related weedy species and spread to the cultivated *Chrysanthemum coronarium*. Given the current high level of concern for non-target damage risks from biological control agents (Follett and Duan 1999; Wajnberg et al. 2000; Louda et al. 2003), it was felt that there would be little possibility of obtaining approval for release of *Napomyza* sp. near *lateralis*. No petition for its release has therefore been submitted.

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7. LIST OF PUBLICATIONS ARISING FROM THE PROJECT

Hinz, H.L., and A.S. McClay. 2000. Ten years of scentless chamomile: prospects for the biological control of a weed of cultivated land. pp. 537-550 *in* Spencer, N.R. (Ed.) Proceedings of the X International Symposium on Biological Control of Weeds, July 4-14, 1999, Bozeman, MT, USA. Montana State University.

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Post-Secondary Education and Training Relevant to Proposal:

Institution	Field Specialization	Degree/Diploma	Year Completed
University of Cambridge	Zoology	Ph.D.	1978
University of Cambridge	Natural Sciences	B.A.	1974

Relevant Professional Experience (begin with present position):

Dates	Position or Function	Employer	Location
1997-	Research Scientist	Alberta Research Council	Vegreville
1984-1997	Research Scientist	Alberta Environmental Centre	Vegreville
1978-1983	Entomologist	Commonwealth Institute of Biological Control	Monterrey, Mexico

Research Activities Related to Research Proposal (list up to 4 projects):

Title	Date
Biological control of *Parthenium hysterophorus*	1978-1983
Biological control of perennial and winter annual weeds in uncultivated land and reduced tillage	1993-1997
Biological control of scentless chamomile	1993-2001
Biological control of false cleavers with a gall mite	1996-1999

Relevant Articles Published in Refereed Journals and Other Relevant Works in the Last Three Years

Craemer, C., R. Sobhian, A.S. McClay, and J.W. Amrine. 1999. A new species of *Cecidophyes* (Acari: Eriophyidae) from *Galium aparine* (Rubiaceae) with notes on its biology and potential as a biological control agent for *Galium spurium*. *International Journal of Acarology* **25**: 255-263.

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Table 1. Releases and recoveries of *Microplontus edentulus* in the prairie provinces, 1997 – 2002.

Release identifier	Stage	Number	Release date	Cage / open	Province	Nearest town	Establish-ment	Recoveries
BC-97-0001	adults	50	Jun 13 1997	O	BC	Hudson's Hope	No	None 1999
BC-98-0001	adults	50	Jun 4 1998	O	BC	Fort St. John	No	None 1998, 1999
AB-97-0009	larvae in plants	c.2000	Jul 22 1997	O	AB	Vegreville	Yes	1998-2002
AB-98-0014	adults	50	Jun 3 1998	O	AB	Spirit River	No	None 1998, 1999, 2000
AB-98-0016	adults	50	Jun 4 1998	O	AB	Edmonton	No	None 1998, 1999
AB-98-0017	adults	75	Jun 5 1998	O	AB	Bruce	No	None 1998, 1999, 2000
AB-98-0015	adults	25	Jun 6 1998	O	AB	Beaverlodge	No	None 1998, 1999, 2000
AB-01-0101	adults	69	Jun 7 2001	C	AB	Edmonton	Possibly	Yes in 2001, none in 2002
SK-98-0002	adults	50	Jun 5 1998	O	SK	Hafford	No	None 1998, 1999
SK-00-M001	adults	50	Jun 9 2000	C	SK	Nipawin	Yes	None in 2001, 18% stems attacked 2002
SK-00-M002	adults	50	Jun 9 2000	C	SK	Coleville	No	None in 2001
SK-01-M003	adults	80	Jun 8 2001	C	SK	Nipawin	Yes	78% stems attacked 2001, 21% stems attacked 2002
SK-01-M004	adults	80	Jun 8 2001	C	SK	Lang	Possibly	54% stems attacked 2001, none in 2002
MB-98-0001	adults	75	Jun 4 1998	O	MB	Winnipeg Beach	No	None 1998

Table 2. Releases of *Rhopalomyia tripleurospermi* in Alberta, 1999, and recoveries. All releases of adults were in 1 m^3 cages. All releases made by transplanting galled plants were uncaged.

Release identifier	Stage	Number	Release date	Near town	Establish-ment	Recoveries
AB-99-0001	adults	1,208♀, 630♂	Apr 22 – June 29, 1999	Vegreville	Yes	1999-2002
AB-99-0004	adults	352	May 27 1999	Bruce	Yes	1999-2002
AB-99-0007	adults	360	Jun 4 1999	Ryley	Yes	1999-2000
AB-99-0020	adults	75♀, 33♂	Jun 14 1999	Bruce	Yes	1999-2000
AB-99-0023	plants	9	Jul 7 1999	Berwyn	Yes	1999-2000
AB-99-0021	adults	200♀,200♂	Jul 8 1999	Bruce	Yes	1999-2000
AB-99-0024	plants	9	Jul 8 1999	Riviere qui Barre	Yes	1999-2000
AB-99-0025	plants	9	Jul 11 1999	Grande Prairie?	Yes	2000
AB-99-0026	plants	9	Jul 11 1999	Beaverlodge	No	none 2000
AB-99-0027	plants	9	Jul 11 1999	Grande Prairie	Yes	2000
AB-99-0022	plants	9	Jul 15 1999	Stony Plain	Yes	1999-2000
AB-99-0028	adults	200♀, 180♂	Jul 15 1999	Stony Plain	Yes	2000
AB-99-0029	adults	200♀,150♂	Jul 16 1999	Hilliard	Yes	2000
AB-99-0030	plants	?	Jul 16 1999	Chipman	No	1999, none 2000
AB-99-0031	adults	40♀,15♂	Jul 16 1999	Mundare	Yes	2000
AB-99-0032	plants	?	Jul 16 1999	Mundare	Yes	1999, 2000
AB-99-0033	plants	?	Jul 16 1999	Chipman	Yes	2000
AB-99-0034	adults	200♀, 150♂	Jul 16 1999	Chipman	No	1999, none 2000
AB-99-0036	plants	9	Jul 27 1999	Nisku	Yes	1999-2000
AB-99-0037	plants	9	Jul 27 1999	Thorsby	Yes	1999-2000
AB-99-0038	plants	9	Jul 27 1999	Warburg	No	none 2000
AB-99-0039	plants	9	Jul 27 1999	Warburg	Yes	1999-2000
AB-99-0040	plants	9	Jul 27 1999	Leduc	Yes	2000
AB-99-0065	plants	5	Jul 27 1999	Leduc	Yes	1999-2000
AB-99-0041	plants	9	Jul 28 1999	Sherwood Park	Yes	2000-2001
AB-99-0042	plants	9	Jul 28 1999	Sherwood Park	No	none 1999-2000
AB-99-0043	plants	7	Jul 28 1999	Musidora	Yes	2002
AB-99-0044	plants		Jul 28 1999	Elk Point	Unknown	1999
AB-99-0067	plants	2	Jul 28 1999	Two Hills	Yes	2002
AB-99-0045	plants	9	Jul 29 1999	Edmonton	Yes	2000
AB-99-0046	plants	9	Jul 29 1999	Edmonton	Yes	1999-2000
AB-99-0047	plants	9	Jul 29 1999	Edmonton	Yes	1999-2000
AB-99-0048	adults	203♀,135♂	Aug 5 1999	Vegreville	No	none 2000

AB-99-0049	adults	306	Aug 9 1999	Vegreville	Yes	1999-2000
AB-99-0050	plants	9	Aug 11 1999	Trochu	Unknown	no data
AB-99-0051	plants	9	Aug 11 1999	Mannville	No	none 2000
AB-99-0052	plants	9	Aug 11 1999	Mannville	No	none 2000
AB-99-0019	plants	9	Aug 13 1999	Vegreville	No	1999, none 2000
AB-99-0055	plants	9	Aug 17 1999	Vegreville	No	1999, none 2000
AB-99-0035	plants	9	Aug 19 1999	Hilliard	No	none 1999
AB-99-0058	plants	9	Aug 20 1999	Edmonton	Unknown	no data
AB-99-0059	plants	9	Aug 20 1999	Edmonton	Yes	1999-2002
AB-99-0060	plants	9	Aug 20 1999	Edmonton	Yes	2000
AB-99-0053	plants	9	Aug 22 1999	Bezanson	Yes	2000
AB-99-0054	plants	9	Aug 22 1999	Bezanson	No	none 2000
AB-99-0056	plants	18	Aug 23 1999	Wetaskiwin	Yes	2000
AB-99-0061	plants	9	Aug 23 1999	Edmonton	Yes	2000
AB-99-0063	plants	9	Sep 1 1999	Carbon	Unknown	no data
AB-99-0064	plants	9	Sep 9 1999	Edmonton	Yes	2000

Table 3. Rearing results with *Microplontus edentulus* in outdoor cage at Vegreville, 1999-2002

Year	Initial no. adults	Date first adults placed on plants	Date first larval emergence	No. larvae collected	No. adults emerged after overwinter storage	Percent overwinter survival
2000	100 ♂ 100 ♀	June 2	July 25	154	89	57.8%
2001	126	June 4	Aug 2	158	84	53.2%
2002	84	May 30	July 17	288	still in storage	--

Table 4. Summary of releases of *Rhopalomyia tripleurospermi* in the prairie provinces, 1999-2002

Year	BC	AB	SK	MB
1999	3	47	3	3
2000	--	7	7	--
2001	--	19	20	--
2002	7	42	14	--

Table 5. Effect of release method on establishment success of *Rhopalomyia tripleurospermi* in Alberta, 1999

Release method	Total no. releases	No. successful	No. unsuccessful	Percent successful
Adults	11	9	2	82%
Transplants	36	26	10	72%
All	47	35	12	74%

Table 6. Effect of release date on establishment success of *Rhopalomyia tripleurospermi* in Alberta, 1999

Release date	Total no. releases	No. successful	No. unsuccessful	Percent successful
up to Jul 15 1999	12	11	1	92%
Jul 16-27, 1999	12	9	3	75%
Jul 28-Aug 11, 1999	11	7	4	64%
Aug 13 - Sep 9, 1999	10	6	4	60%

Table 7. Measures of insect abundance in cages assigned to "with" and "without" treatments for each insect species, field experiment, Vegreville, 2000. Means ± standard error. Significance of treatment effects from 2-way ANOVA for blocks and insect treatment.

		Plant 1		Plant 3	
Insect species	Measure	with	without	with	without
R. tripleurospermi	total galls per plant	173.8 ± 32.1 p<0.0001	19.8 ± 7.7	18.7 ± 2.8 NS	12.7 ± 3.3
M. edentulus	exit holes per plant	23.7 ± 8.6 p=0.0155	0.0 ± 0.0	10.4 ± 1.9 p<0.0001	0.9 ± 0.4
O. hookeri	total adults emerged	22.5 ± 8.9 NS	43.1 ± 20.8	62.8 ± 12.1 NS	58.0 ± 13.2

Table 8. Effects of *R. tripleurospermi* and *M. edentulus* on scentless chamomile performance, field experiment, Vegreville 2000. No significant treatment effects.

	Plant 1		Plant 3	
Insect	without	with	without	with
R. tripleurospermi				
Number of stems	25.5 ± 1.8	24.9 ± 1.6		
Number of flowers	425.8 ± 102.4	324.9 ± 97.5		
Mean stem height (cm)	102.5 ± 9.5	93.5 ± 7.0		
Total stem height (cm)	2794 ± 334	2255 ± 178		
Shoot biomass (g)	241.9 ± 28.2	222.3 ± 18.8		
Number of seeds	13631 ± 6122	5895 ± 3009		
M. edentulus				
Number of stems	25.1 ± 1.2	25.2 ± 2.0	8.6 ± 0.4	8.9 ± 0.4
Number of flowers	420.3 ± 99.0	336.3 ± 101.6	21.3 ± 7.6	32.8 ± 13.5
Mean stem height (cm)	100.8 ± 8.7	95.2 ± 8.1	87.1 ± 3.9	80.1 ± 3.6
Total stem height (cm)	2593 ± 249	2456 ± 301	748 ± 53	704 ± 33
Shoot biomass (g)	243.9 ± 26.5	220.3 ± 27.3	21.4 ± 1.1	22.9 ± 2.5
Number of seeds	15459 ± 6148	4067 ± 2532	3963 ± 832	3451 ± 1093

Table 9. Measures of insect abundance in cages assigned to "with" and "without" treatments for each insect species, field experiment, Vegreville, 2001. Means ± standard error. Significance of treatment effects from 2-way ANOVA for blocks and insect treatment.

Insect species	Measure	with	without	
R. tripleurospermi	total galls per plant	66.0 ± 10.2	7.6 ± 2.0	p<0.0001
M. edentulus	exit holes per plant	30.6 ± 7.8	1.6 ± 1.6	p=0.0006
O. hookeri	total adults emerged	440.1 ± 111.2	21.9 ± 9.4	p=0.0005

Table 10. Analysis of variance for insect effects in 2001 field plot impact experiment. Only main effects significant at $p<0.05$ shown.

Insect treatment	Response variable	DF	SS	MS	F	p
R. tripleurospermi	mean stem height	1	684.038	684.038	4.91	0.0360
	number of flowers	1	1980050	1980050	10.35	0.0036
O. hookeri	number of flowers		861328	861328	4.50	0.0439
	number of seed heads	1	1334161	1334161	8.21	0.0083
	total seed weight	1	9148.56	9148.56	5.94	0.0223
M. edentulus	number of seed heads	1	825613	825613	5.08	0.0332

Table 11. Least squares means for treatment effects in 2001 field plot impact experiment. Only main effects significant at $p<0.05$ shown.

Insect treatment	Response variable	Mean without insect	Mean with insect	Standard error
R. tripleurospermi	mean stem height (cm)	115.0	105.8	3.0
	number of flowers	1,327.8	830.3	110.3
C. hookeri	number of flowers	1,243.1	914.9	110.3
	number of seed heads	918.3	1,326.6	97.3
	total seed weight (g)	113.8	80.0	10.0
M. edentulus	number of seed heads	961.8	1,283.1	97.3

Table 12. Stem height, number of stems and total biomass of surviving plants from 50 galled and 50 ungalled plants marked in the field, November 16, 2001, and harvested July 12 2002. Significant differences between groups tested by Student's t.

	Mean for ungalled plants (n=29)	Mean for galled plants (n=24)	
No. stems	1.00 ± 0.00	1.83 ± 0.22	$p<0.001$
Mean stem height (cm)	14.4 ± 1.4	12.4 ± 1.3	NS
Total biomass (g) excluding old gall	0.403 ± 0.054	0.622 ± 0.114	NS
No. flowers	1.3 ± 0.29	2.0 ± 0.42	NS

Table 13. Stem height, number of stems and total biomass of 30 galled and 29 ungalled scentless chamomile plants overwintered outdoors as rosettes, transplanted to the field on May 9 2002, and harvested on July 4 2002. Ungalled and galled groups compared by one-way analysis of variance.

	Mean for ungalled plants (n=29)	Mean for galled plants (n=30)	
No. stems	6.0 ± 0.42	11.8 ± 0.90	$p<0.001$
Mean stem height (cm)	37.0 ± 1.9	27.4 ± 1.0	$p<0.001$
Total biomass (g)	21.1 ± 2.6	19.2 ± 1.7	NS
No. flowers	21.3 ± 4.5	17.8 ± 3.3	NS

Table 14. Dispersal of *Omphalapion hookeri* and *Rhopalomyia tripleurospermi* from their release sites at Vegreville, 1997-2002.

Year	Omphalapion hookeri Released 1993		*Rhopalomyia tripleurospermi* Released 1999	
	Distance of spread (km)	Area occupied (km^2)	Distance of spread (km)	Area occupied (km^2)
1996	0.18	0.03		
1997	0.8	0.4		
1998	5.5	6.8		
1999	7.1	22	0.12	0.03
2000	10.3	116	1.8	3.2
2001	14.7	230	7.2	42
2002	15.6	116	15.6	250

Table 15. Results of no-choice adult longevity, feeding and oviposition tests with *Napomyza* sp. near *lateralis* in 1999.

Plant species	No. replicates	Mean longevity ± SE (days)	Mean no. feeding holes/♀/day	Mean no. eggs laid/♀/day
Anthemideae				
Tripleurospermum perforatum	33	11.2 ± 1.1	62.4 ± 6.1	1.5 ± 0.2
Matricaria recutita	8[a]	7.9 ± 0.7	27.3 ± 6.4	0.43 ± 0.23
Chamaemelum nobile	8[a]	7.4 ± 1.5	3.4 ± 1.8	0
Anthemis sancti-johannis	5	9.0 ± 1.6	45.5 ± 17.9	0.06 ± 0.04
A. tinctoria	5	7.4 ± 0.8	12.8 ± 4.4	0.15 ± 0.06
Tanacetum parthenium	8	5.6 ± 0.6	20.1 ± 9.4	0.06 ± 0.06
T. vulgare	5	7.0 ± 0.6	29.8 ± 8.2	0.14 ± 0.14
Achillea millefolium	5	6.6 ± 0.4	3.3 ± 1.7	0
Chrysanthemum carinatum	7	9.4 ± 2.9	79.3 ± 18.1	0.25 ± 0.17
C. coronarium 'Big Leaf'	12[a]	8.8 ± 1.8	38.7± 6.9	0.20 ± 0.13
Coleostephus myconis	8[a]	9.3 ± 2.5	60.5 ± 12.0	0.21 ± 0.11
Leucanthemum vulgare	5	7.4 ± 0.2	7.9 ± 3.2	0
Dendranthema grandiflora 'Yellow Diamond' and 'Sierra'	7[a]	7.4 ± 0.8	1.7 ± 1.5	0
Lonas annua	11[a]	6.0 ± 0.3	7.1 ± 2.2	0
Heliantheae				
Helianthus annuus *ssp.* lenticularis	8[a]	5.6 ± 0.3	0.85 ± 0.44	0
Cardueae				
Cirsium arvense	6	6.5 ± 0.4	0	0
Acroptilon repens	5	8.2 ± 0.8	23.0 ± 4.4	0.24 ± 0.11

[a], some replicates with choice between leaf and shoot with flower bud.

Table 16. Results of no-choice development tests with *Napomyza* sp. near *lateralis* in 1999.

Tribe Plant species	Stage *	No. plants offered	Total no. shoots	Number of individuals found upon dissection			
				Larvae	Pupae	**Total**	Mean ± SE
Anthemideae							
Tripleurospermum perforatum CAN tetrapl.	Bo-Bu	6	31	10	68	**78**	13.0 ± 5.5
T. perforatum CAN dipl.	Bo-Bu	15	57	51	342	**393**	26.2 ± 3.1
T. perforatum Austria	Bo-Fl	10	59	9	161	**170**	17.0 ± 4.4
T. perforatum RV	Bo-Bu	6	52	49	98	**147**	24.5 ± 6.6
T. ambiguum	R-Bu	4	24	2	75	**77**	19.3 ± 4.5
Matricaria recutita	R-Bu	4	54	2	40	**42**	10.5 ± 4.3
M. discoidea	R-Bo	3	40	17	17	**34**	11.3 ± 3.9
Anthemis sancti-johannis	Bo-Bu	4	19	1	2	**3**	0.8 ± 0.5
A. tinctoria	Bo-Bu	4	22	9	10	**19**	4.8 ± 2.8
T. cinerariifolium	Bo-Bu	2	11	0	0	**0**	0
Tanacetum huronense	Bo	2	8	0	0	**0**	0
T. parthenium	Bo	3	25	2	2	**4**	1.3 ± 1.3
T. vulgare	Bo-Bu	5	50	1	1	**2**	0.4 ± 0.2
Artemisia cana	Bo	3	3[a]	0	0	**0**	0
Artemisia frigida	Bo	2	13	0	0	**0**	0
A. vulgaris	Bo	2	12	0	0	**0**	0
Coleostephus myconis	Bo-Bu	2	2[a]	0	2[b]	**2[b]**	
Chrysanthemum coronarium 'Big Leaf'	Bu	2	2[a]	2	18	**20**	
Dendranthema indica	Bo-Fl	3	14	0	0	**0**	0
D. grandiflora 'Sierra'	Bo	2	6	0	0	**0**	0
'Mertense Bottomley'	Bo-Fl	4	23	0	0	**0**	0
'Garnet Burgund'	Bo-Bu	5	20	0	0	**0**	0
'Norden Canary'	Bo-Bu	6	50	0	0	**0**	0
'Yellow Diamond'	Bo	2	5	0	0	**0**	0
Leucanthemum maximum	Bo	2	11	0	0	**0**	0
L. vulgare	Bo-Fl	3	15	0	4	**4**	1.3 ± 0.1
Achillea filipendulina	Bo	3	17	0	0	**0**	0
A. millefolium	Bu-Fl	2	17	0	0	**0**	0
A. ptarmica	Bo-Bu	3	27	0	0	**0**	0
Cotula sp.	Bo-Fl	1	25	0	0	**0**	0
Lonas annua	Bo-Bu	2	2[a]	1	0	**1**	
Sphaeromeria capitata	Ro-Bo	4	13	0	0	**0**	0
Heliantheae							

Bidens cernuus	Bo	1	1[a]	0	0	0
Echinacea purpurea	R	4	4	0	0	0
Helianthus annuus 'Cargill'	Bo	4	4	0	0	0
H. annuus spp. *lenticul.*	Bu	2	2	0	1	1
H. nuttallii	Bo	2	36	0	0	0
Rudbeckia hirta	Bo	2	14	0	0	0
Astereae						
Aster novae-angliae	Bo	2	15	0	0	0
Erigeron annuus	R-Bo	4	15	0	0	0
E. caespitosus	Bo-Fl	3	14	0	0	0
Grindelia squarrosa	Bo	2	18	0	0	0
Senecioneae						
Senecio eremophilus	Bo	2	6	0	0	0
S. vulgaris	Bu	5	5	0	0	0
Cichorieae						
Lactuca sativa	R-Bo	3	2	0	0	0
Taraxacum officinale	Bu-Fl	2	4	0	0	0
Inuleae						
Helichrysum arenarium	Bu	2	2[a]	0	0	0
Inula ensifolia	Bo	5	18	0	0	0
Cardueae						
Carduus nutans	Bu	2	3	0	0	0
Centaurea jacea	R-Bo	1	8	0	0	0
C. solstitialis	Bu	2	8	0	0	0
Cirsium arvense	Bo	4	18	0	0	0
Helenieae						
Eriophyllum lanatum	Bo	6	8[a]	0	0	0

*R – rosette, Bo – bolting, Bu – budding, Fl – flowering.

[a], plants with only one main shoot but many secondary shoots (branched); [b], adults already emerged, i.e. no verification that these were really *Napomyza* sp. near *lateralis*.

Table 17. Results of single-choice development tests with *Napomyza* sp. near *lateralis* in 1999.

Plant species	No. replicates	Total no. shoots	Total no. of flies released	Number of individuals found upon dissection			
				Larvae	Pupae	**Total**	Mean ± SE
Matricaria recutita	5	39	20 ♀	2	38	**40**	8.0 ± 1.2
Control	5	36	10 ♂	42	146	**188**	37.6 ± 6.9
Lonas annua	3	3[a]	6 ♀	2[b]	0	**2**	0.7 ± 0.7
Control	3	16	3 ♂	18	79	**97**	32.3 ± 11.9
Anthemis sancti-johannis	3	12	6 ♀	1[b]	0	**1**	0.3 ± 0.3
		---[c]	2 ♂				
Control	2			0	7	**7**	3.5
Chrysanthemum coronarium 'Big Leaf'	3	6	6 ♀	0	0	**0**	0
			3 ♂				
Control	3	3		7	17	**24**	8.0 ± 1.5
Helianthus annuus ssp. *lenticularis*	2	2	8 ♀	0	0	**0**	0
			4 ♂				
Control	2	8		11	56	**67**	33.5
Bidens cernuus	2	5	8 ♀	0	0	**0**	0
Control	2	4	4 ♂	19	186	205	102.5

[a], plant branched; [b], larvae dead; [c], plants stayed in rosette stage.

Table 18. Results of field cage test carried out with *Napomyza* sp. near *lateralis* in 1999.

Plant species	No. plants offered	Total no. shoots	Number of individuals found upon dissection			
			Larvae	Pupae	Total	Mean ± SE
Anthemideae						
Tripleurospermum perforatum CAN tetraploid	3	15	1	18	**19**	6.3 ± 1.5
T. perforatum Austria	3	23	7	53	**60**	20.0 ± 3.2
Chamaemelum nobile	3	32	0	0	**0**	0
Tanacetum parthenium	3	22	0	0	**0**	0
Chrysanthemum carinatum	3	26	0	1	**1**	0.3 ± 0.3
C. coronarium 'Big Leaf'	3	20	0	0	**0**	0
Lonas annua	3	24	0	0	**0**	0
Heliantheae						
Bidens cernuus	3	3[a]	0	0	0	0

[a], plant branched.

Table 19. Results of open-field test with *Napomyza* sp. near *lateralis* in 2000. All plants exposed are in the tribe Anthemideae. Attack levels of plants were pooled over all plots.

Plant species	No. plants exposed	% plants attacked	Average number of *Napomyza* individuals found upon dissection (± SE)
Tripleurospermum perforatum	32	100	7.2 ± 0.7 a
Anthemis cotula	20	70	2.1 ± 0.5 b
Anthemis tinctoria	18	33.3	0.5 ± 0.9 c
Chrysanthemum carinatum	20	45	0.75 ± 1.2 bc
Tanacetum parthenium	20	5[a]	0.05 ± 0.05 c

[a], only one mine found (see text for details). Attack levels were compared using a Tamhane test ($P < 0.05$) for samples with unequal variances. Means followed by the same letter are not significantly different.

Table 20. Results of continuation trial with *Napomyza* sp. near *lateralis* in 2000.

Plant species	No. cages setup	No. plants exposed	No. ♀ released	No. adults produced in subsequent generations		Development time (days)	
				Total	Mean per ♀ ± SE[a]	Mean ± SE[a]	Range
1st generation							
Anthemideae							
Tripleurospermum perforatum	1	5	15	107 (58♀ + 49♂)	**7.1**	64	46 - 97
Chrysanthemum coronarium 'Big Leaf'	1	5	20	217 (98♀ + 119♂)	**10.9**	50	46 - 68
C. carinatum	1	5	15	89 (50♀ + 49♂)	**5.9**	57	46 - 78
Anthemis sancti-johannis	1	5	15	64 (40♀ + 24♂)	**4.3**	65	48 - 90
A. tinctoria	1	2	10	23 (15♀ + 8♂)	**2.3**	65	49 - 82
Tanacetum parthenium	1	4	10	21 (14♀ + 7♂)	**2.1**	76	63 - 93
Heliantheae							
Helianthus annuus ssp. lenticularis	1	5	15	2[b]	**0.13**		
2nd generation							
Tripleurospermum perforatum	2	10	42	420 (202♀ + 218♂)	**10.0**	55	43 - 71
Chrysanthemum coronarium 'Big Leaf'	2	13	57	146 (86♀ + 60♂)	**2.6**	54	43 - 64
C. carinatum	2	10	41	198 (106♀ + 92♂)	**4.8**	61	48 - 88
Anthemis sancti-johannis	1	4	30	1 (♀)	**0.03**	(92)	---
A. tinctoria	1	3	15	5 (3♀ + 2♂)	**0.3**	75	73 - 87
T. parthenium	1	2	12	0	**0**	---	---

3rd generation							
Tripleurospermum perforatum	8	38	238	1,244 (646♀ + 598♂)	**5.3 ± 0.9** a	60 ± 2.4 a	44 - 95
Chrysanthemum coronarium 'Big Leaf'	3	23	79	374 (178♀ + 196♂)	**4.8 ± 0.5** a	56 ± 1.5 a	40 - 87
C. carinatum	3	13	49	37[b] (11♀ + 10♂)	**0.8 ± 0.1** b		
Anthemis sancti-johannis	1	2	1	0	**0**	---	---
A. tinctoria	1	1	3	0	**0**	---	---

[a], SE only given for species with more than two replicates (one cage was treated as one replicate); [b], including pupae and larvae (see text for details). The average number of offspring produced per female on *T. perforatum*, *C. coronarium* and *C. carinatum* during the fourth generation was compared using a Tukey HSD-test ($P < 0.05$) after log10-transformation of data. Average development time was compared with an independent samples t-test. Means followed by the same letter are not significantly different.

Table 21. Results of cross-breeding experiments carried out with *Napomyza* sp. near *lateralis* originating from *Tripleurospermum perforatum* and *Acroptilon repens* in 2000.

♀ x ♂	Tripleurospermum perforatum *(T)*		Acroptilon repens *(A)*	
	No. replicates	Average attack per plant (± SE)[a]	No. replicates	Average attack per plant (± SE)[a]
A x A			24	0.54 ± 0.12
A x T			18	0.3 ± 0.11
T x A	5	0	9	0

[a], Number of larvae and pupae found upon dissection.

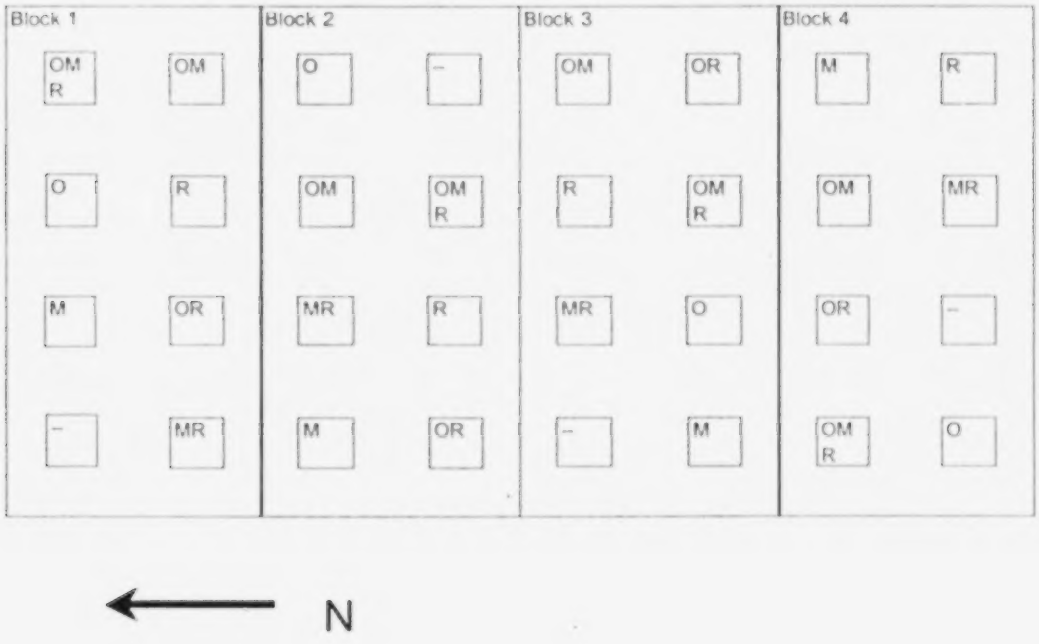

Figure 1 Layout of field cage impact evaluation experiments in 2000 and 2001. Treatments: O, *Omphalapion hookeri*; M, *Microplontus edentulus*; R, *Rhopalomyia tripleurospermi*.

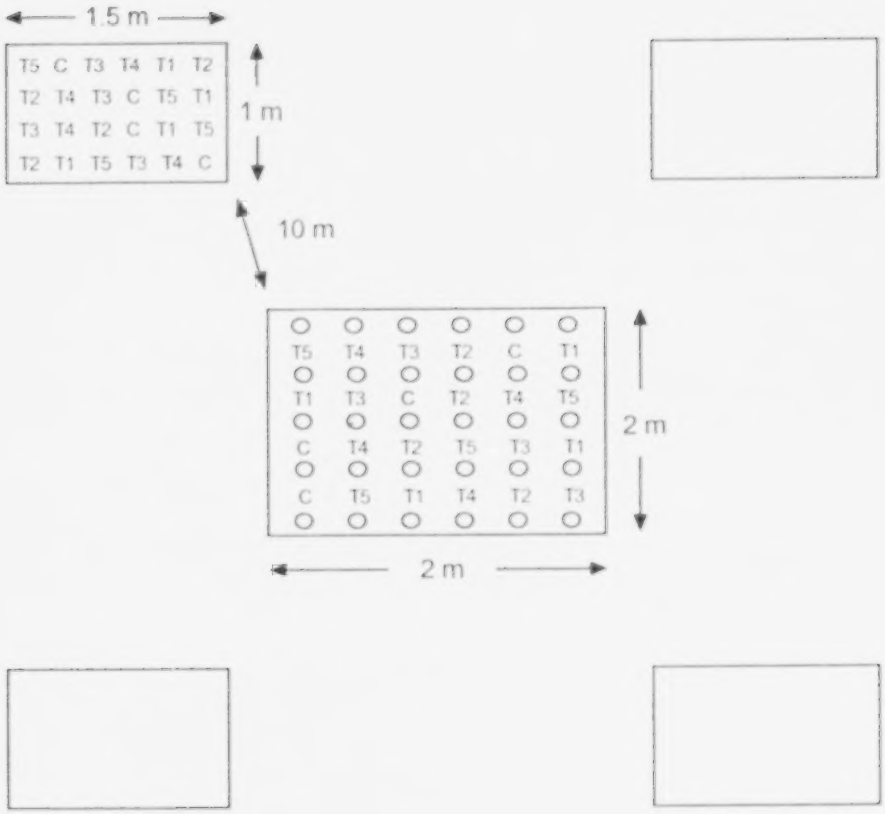
1.5 m
T5 C T3 T4 T1 T2
T2 T4 T3 C T5 T1
T3 T4 T2 C T1 T5
T2 T1 T5 T3 T4 C
1 m
10 m
T5 T4 T3 T2 C T1
T1 T3 C T2 T4 T5
C T4 T2 T5 T3 T1
C T5 T1 T4 T2 T3
2 m
2 m

Figure 2 Design of open-field test with *Napomyza* sp. near *lateralis* at CABI Bioscience Switzerland Centre in 2000. C, control (*Tripleurospermum perforatum*); T1- T5, test plant species: *Matricaria recutita, Anthemis cotula, A. tinctoria, Chrysanthemum carinatum,* and *Tanacetum parthenium;* O , infested *T. perforatum* control plants.

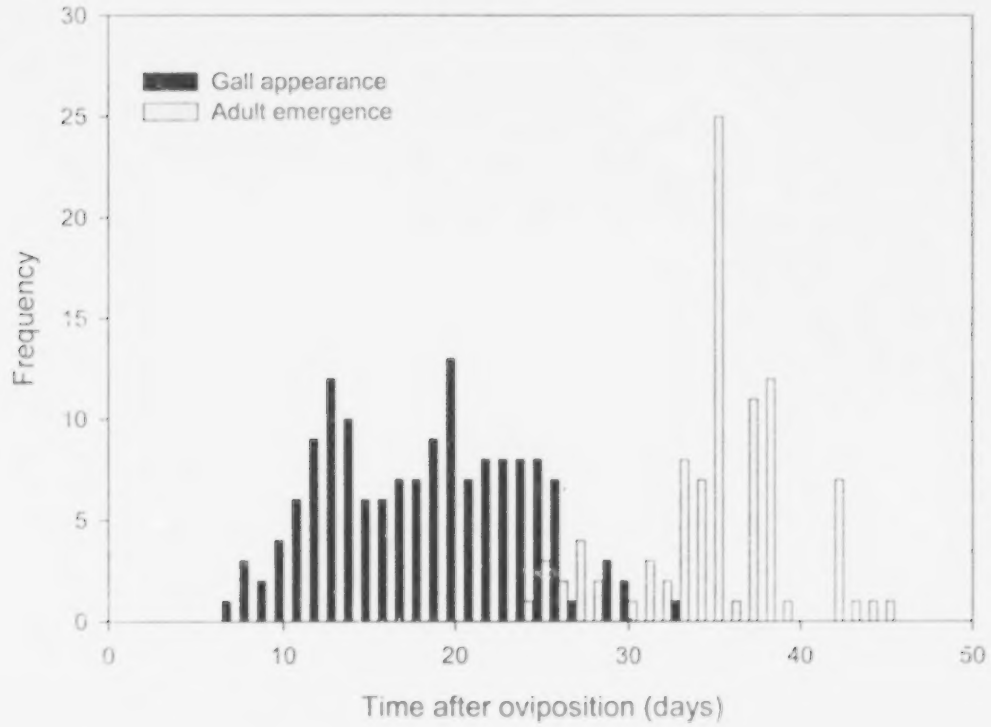

Figure 3 Time from oviposition to appearance of galls and emergence of adults in greenhouse colony of *Rhopalomyia tripleurospermi*, Vegreville, 1999.

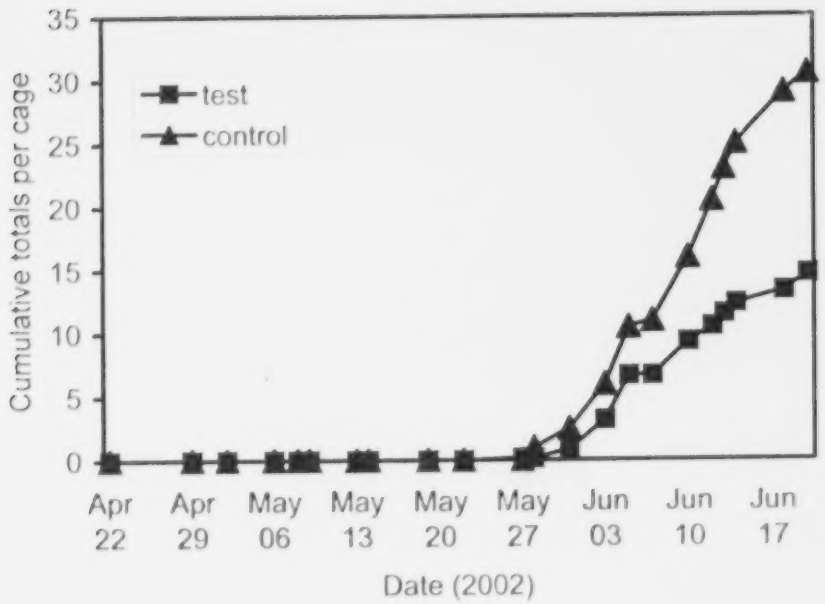

Figure 4 Cumulative counts of adult *Omphalapion hookeri* on scentless chamomile plants in overwinter survival test cages, spring 2002.

Figure 5 Dispersal of *Omphalapion hookeri* from its 1993 release site at Vegreville, 1997-2002

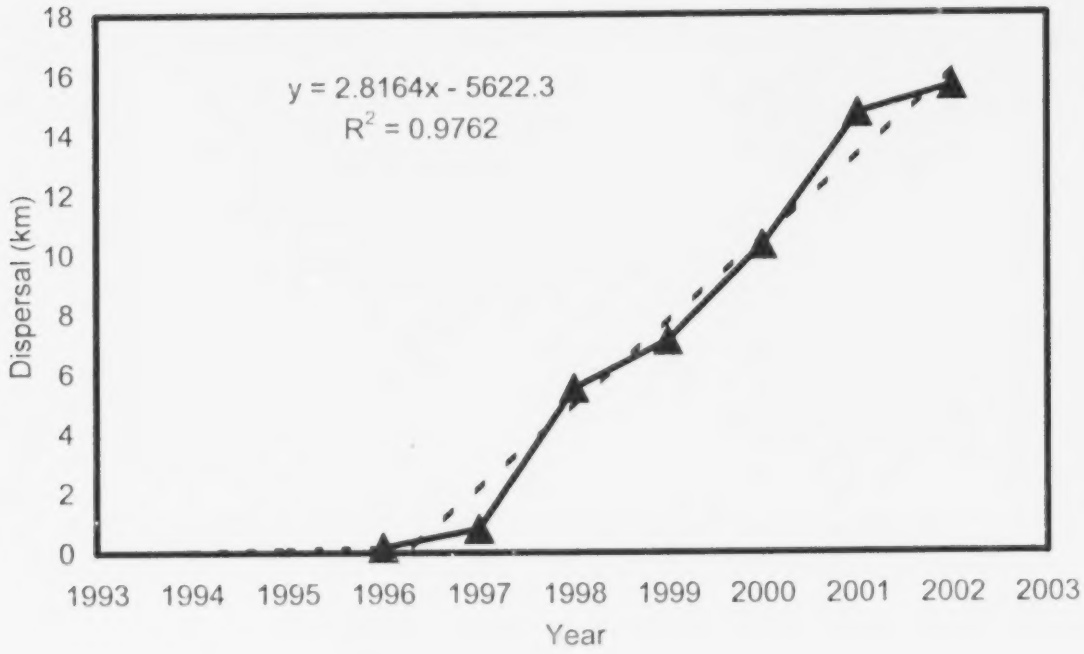

Figure 6 Distances of spread of *Omphalapion hookeri* from the 1993 Vegreville release site, 1996-2002

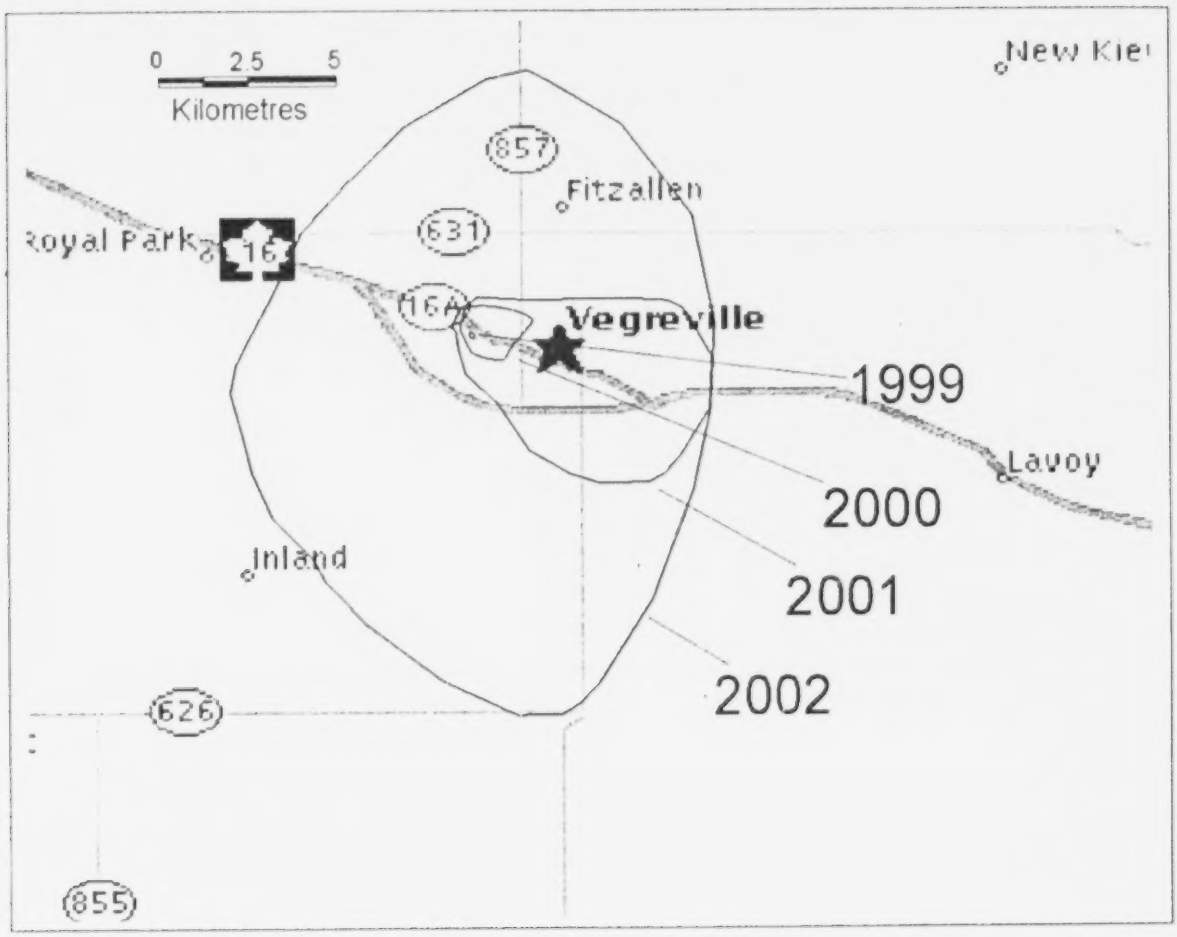

Figure 7 Dispersal of *Rhopalomyia tripleurospermi* from the 1999 Vegreville release site, 1999-2002.

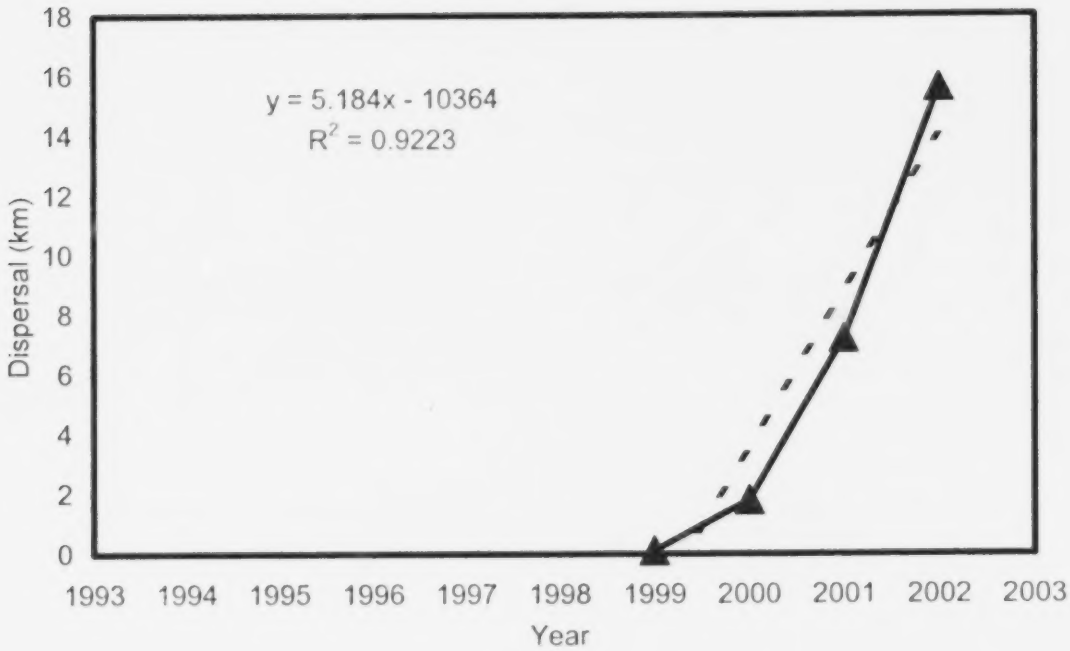

Figure 8 Distances of spread of *Rhopalomyia tripleurospermi* from the 1999 Vegreville release site, 1999-2002

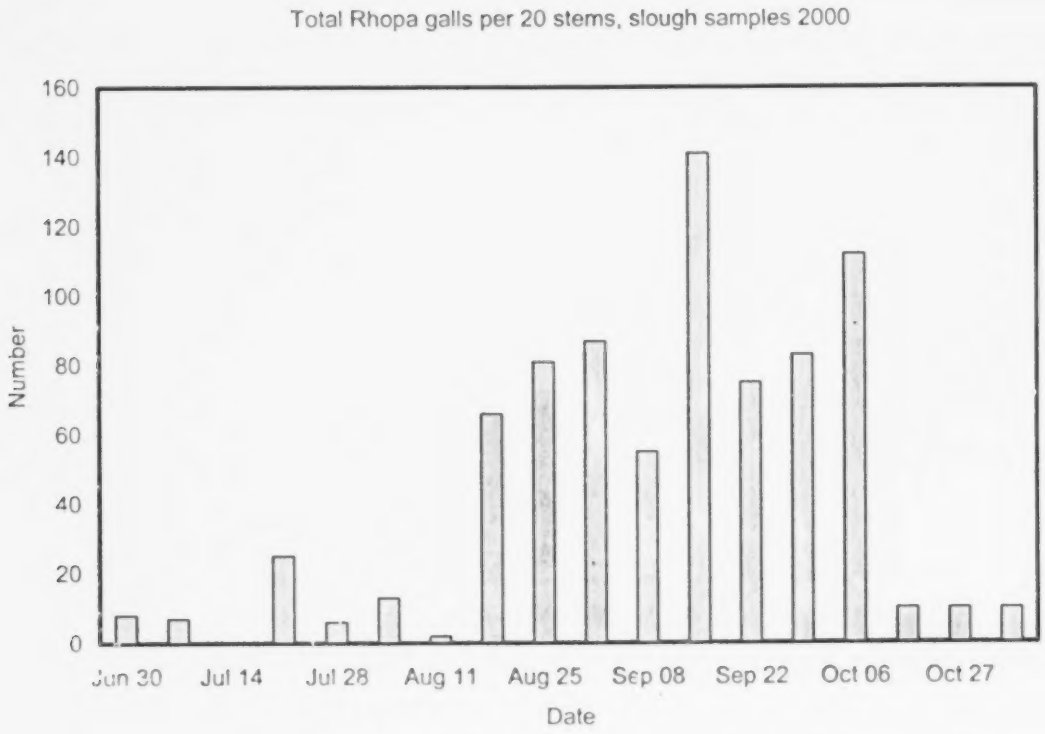

Figure 9 Numbers of *Rhopalomyia tripleurospermi* galls per 20 scentless chamomile stems, slough site, ARC Vegreville, summer 2000.

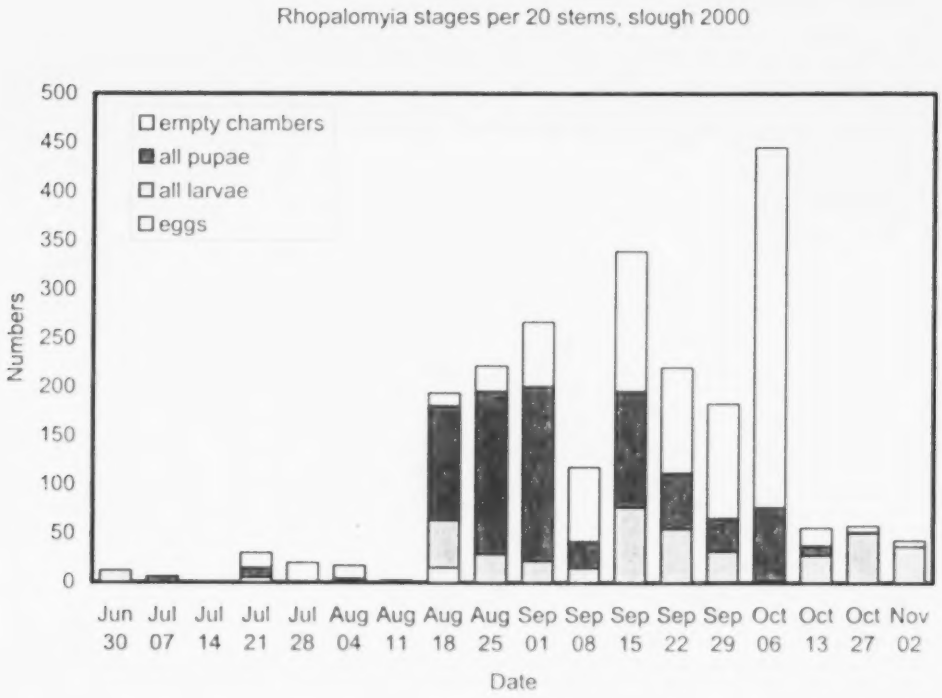

Figure 10 Stages of *Rhopalomyia tripleurospermi* in samples of 20 scentless chamomile stems, slough site, ARC Vegreville, summer 2000.

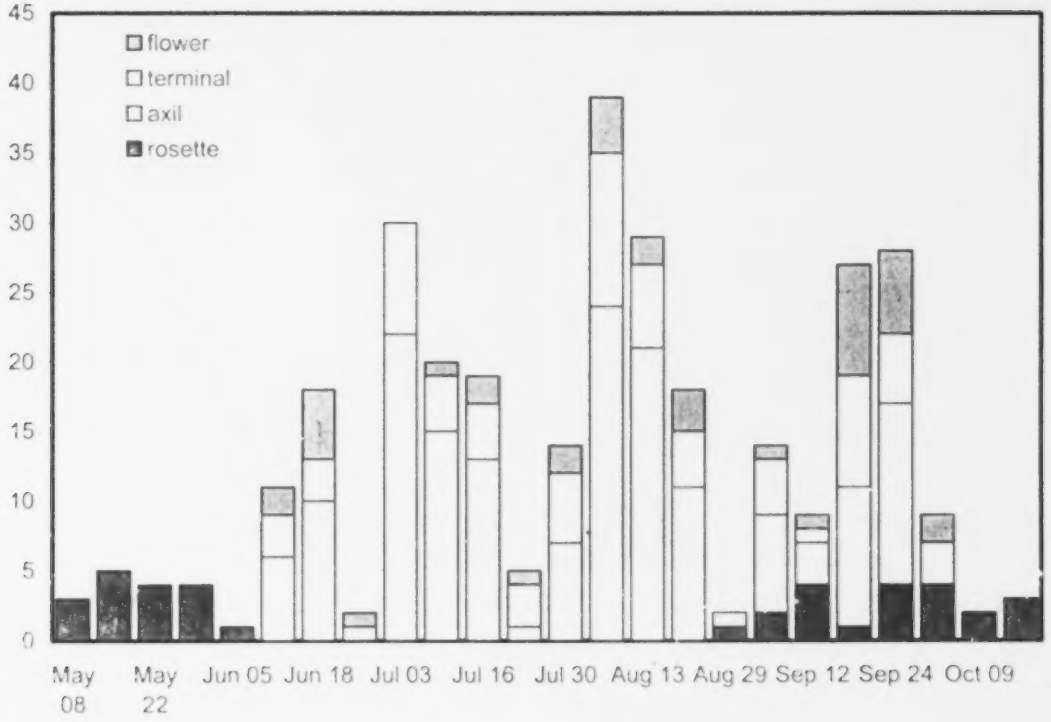

Figure 11 Numbers and types of *Rhopalomyia tripleurospermi* galls in samples of 20 scentless chamomile stems, slough site, ARC Vegreville, 2001.

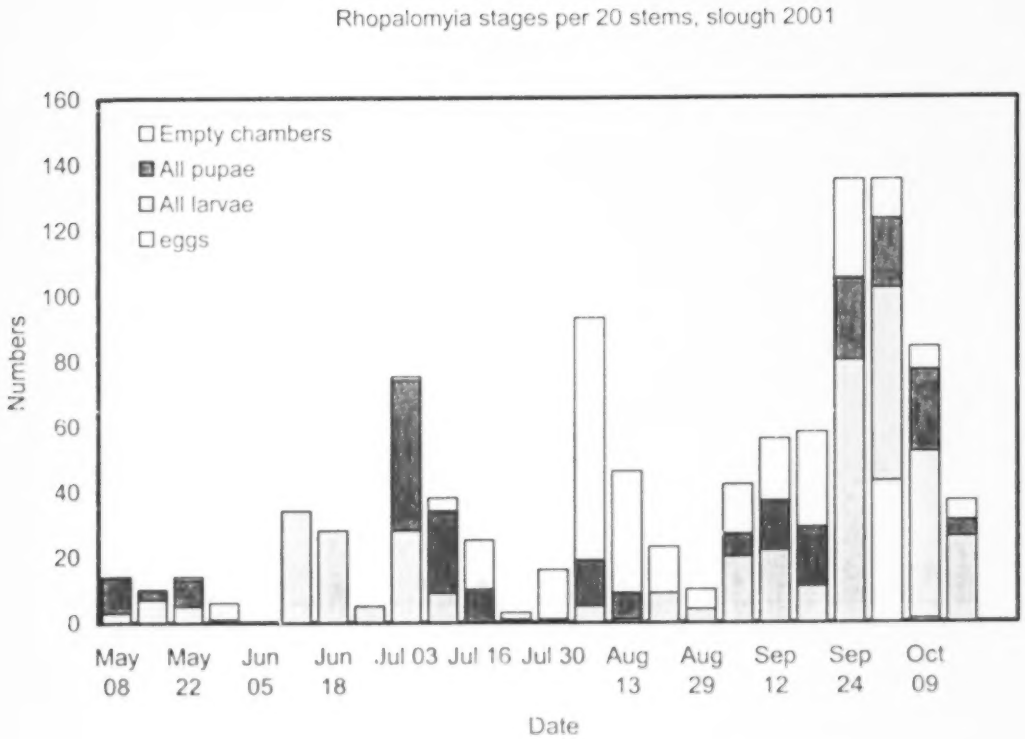

Figure 12 Stages of *Rhopalomyia tripleurospermi* found by dissection of weekly samples of 20 galled stems, slough site, ARC, Vegreville, Alberta, summer 2001.

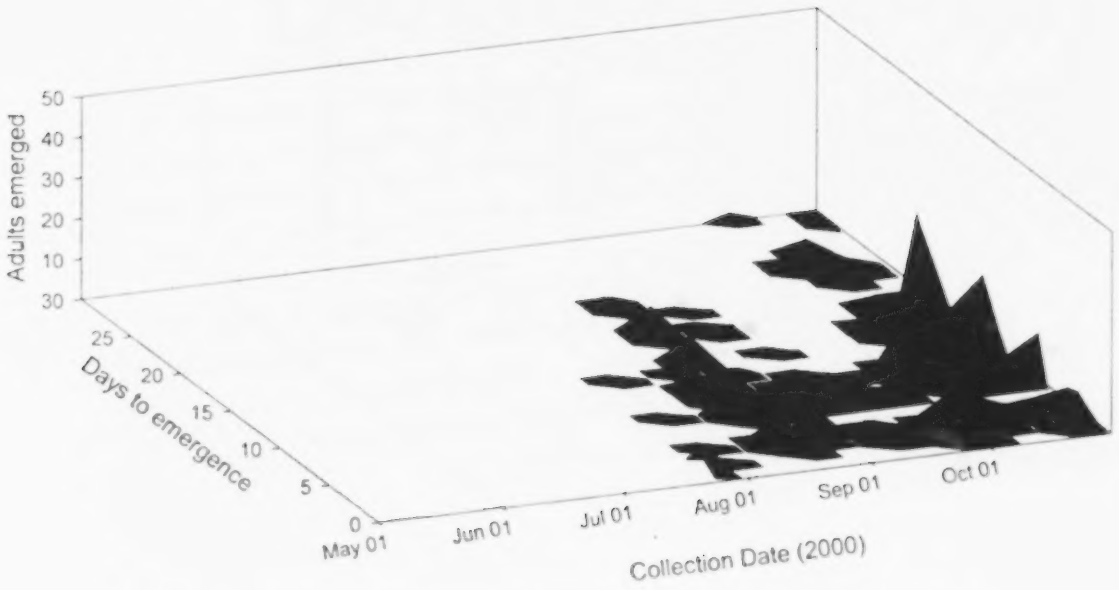

Figure 13 Emergence of *Rhopalomyia tripleurospermi* adults from samples of 20 field-collected galls, Vegreville, 2000, plotted by collection date and time to emergence.

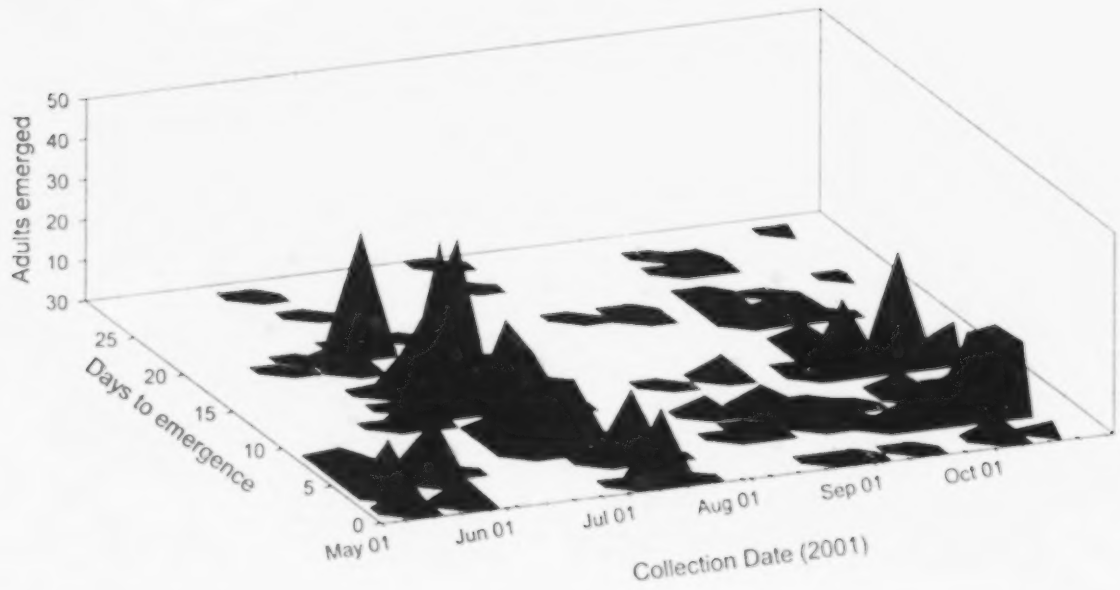

Figure 14 Emergence of *Rhopalomyia tripleurospermi* adults from samples of 20 field-collected galls, Vegreville, 2001, plotted by collection date and time to emergence.

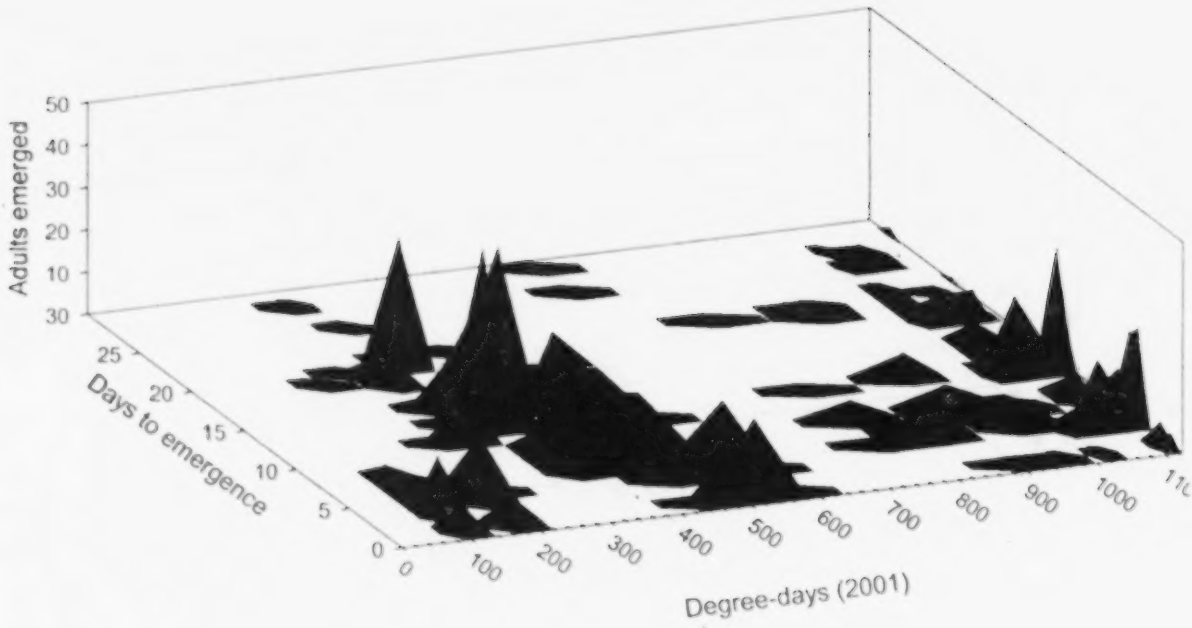

Figure 15 Emergence of *Rhopalomyia tripleurospermi* adults from samples of 20 field-collected galls, Vegreville, 2001, plotted by accumulated degree-days and time to emergence.

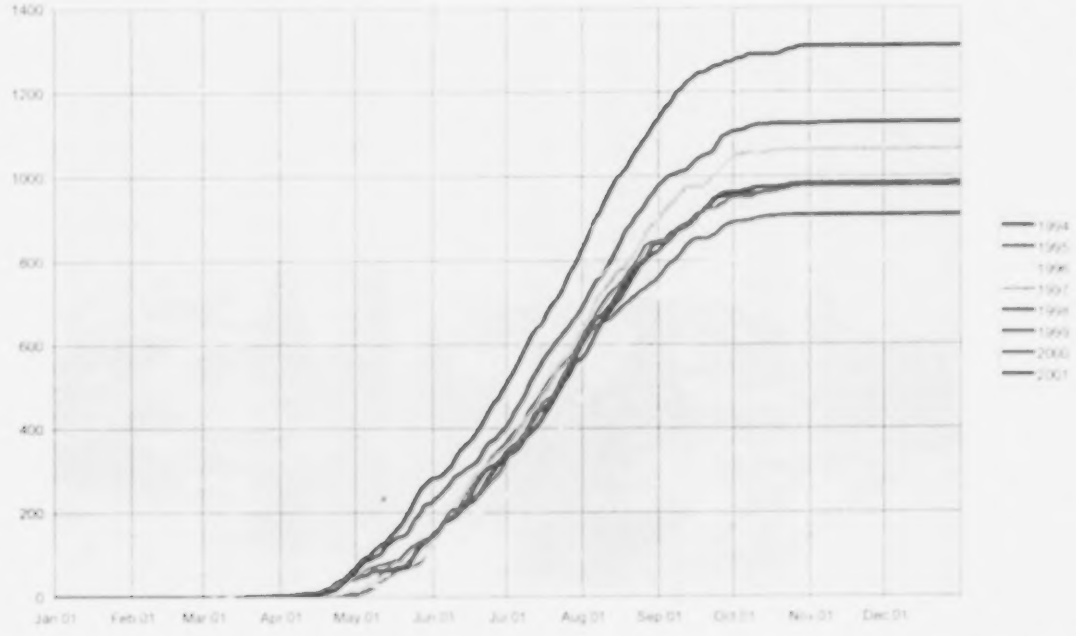

Figure 16 Accumulated degree-days above 7.7°C from January 1, 1994 – 2001, Vegreville, Alberta.

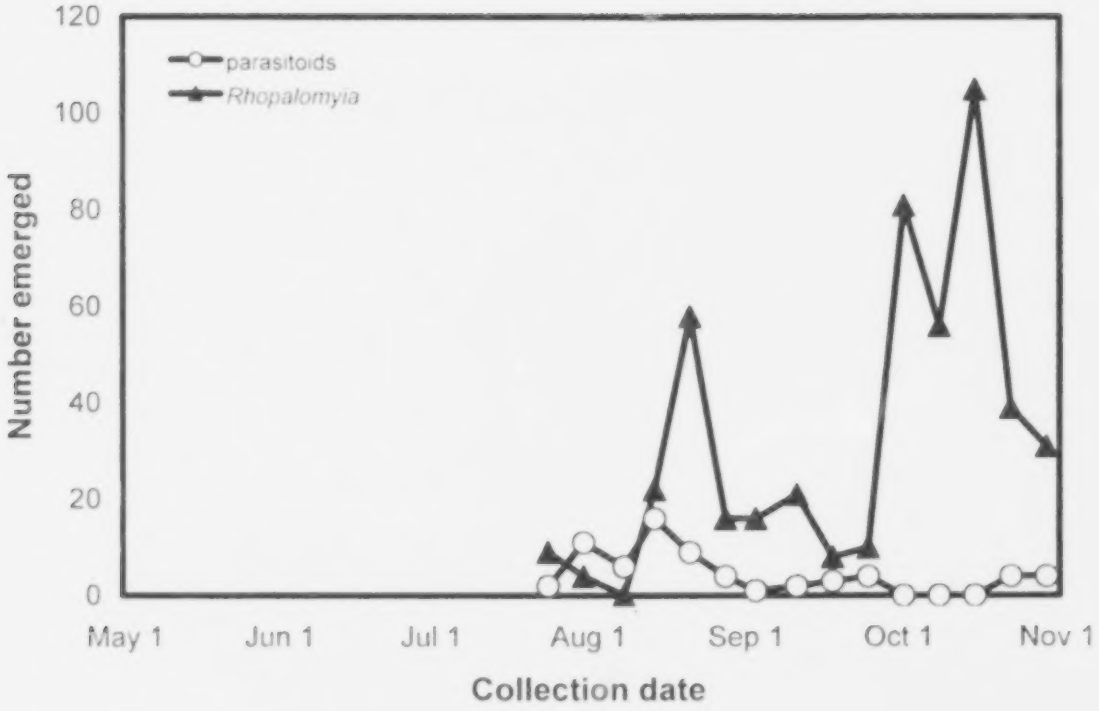

Figure 17 Emergence of adult *Rhopalomyia tripleurospermi* and parasitoids from weekly samples of 20 field-collected galls, Vegreville, Alberta, summer 2000.

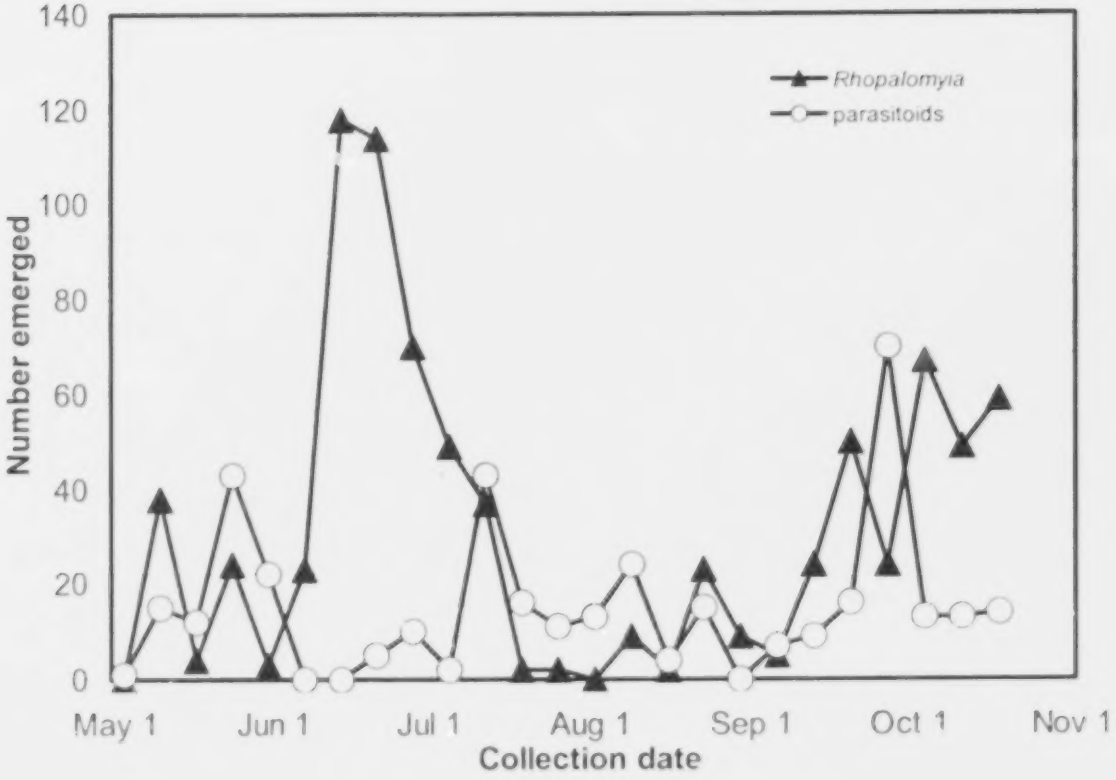

Figure 18 Emergence of adult *Rhopalomyia tripleurospermi* and parasitoids from weekly samples of 20 field-collected galls, Vegreville, Alberta, summer 2001.

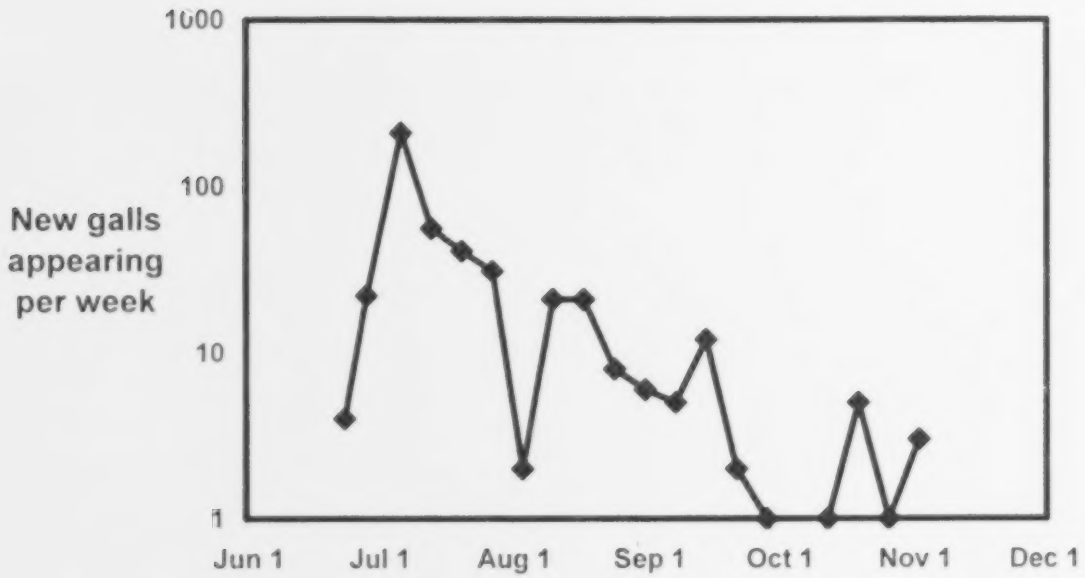

Figure 19 Numbers of new *Rhopalomyia tripleurospermi* galls appearing per week on release plots, ARC, Vegreville, Alberta, summer 2000.

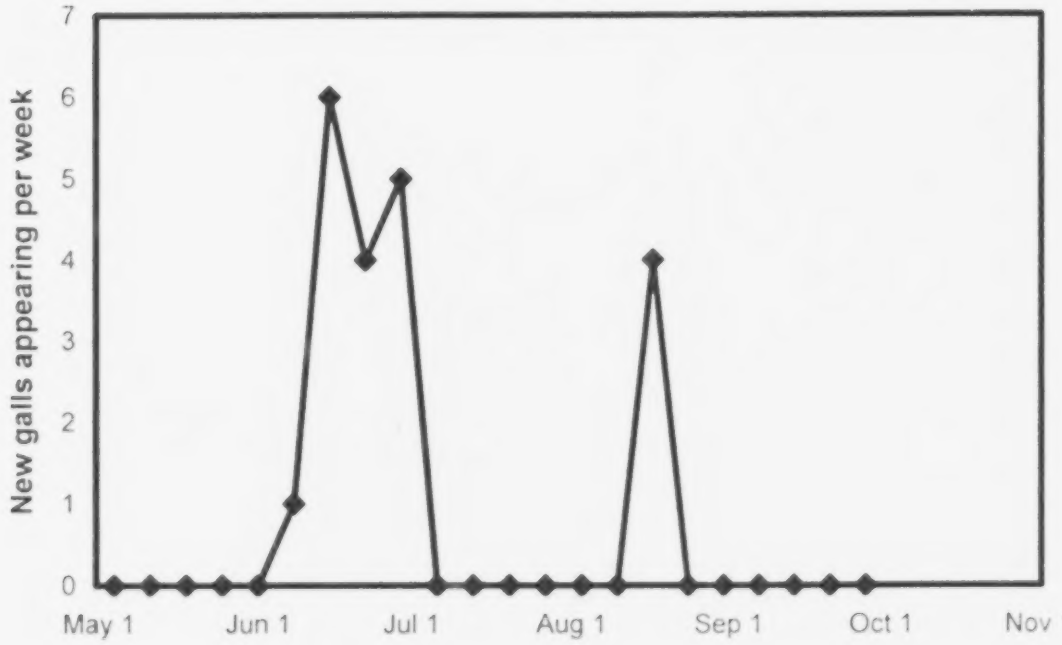

Figure 20 Numbers of new *Rhopalomyia tripleurospermi* galls appearing per week on release plots, ARC, Vegreville, Alberta, summer 2001.

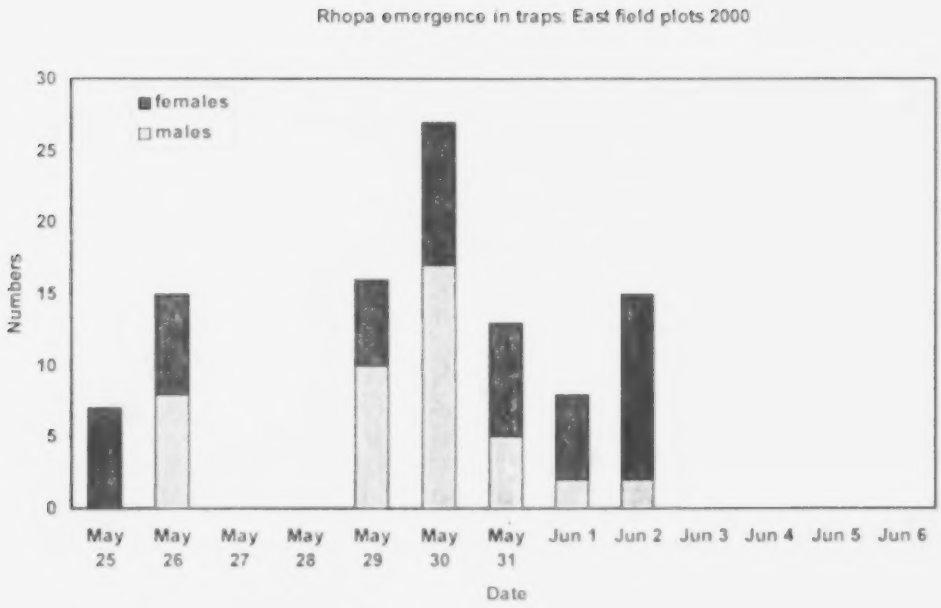

Figure 21 Emergence of *Rhopalomyia tripleurospermi* adults from overwintered rosettes, Vegreville, Alberta, spring 2000.

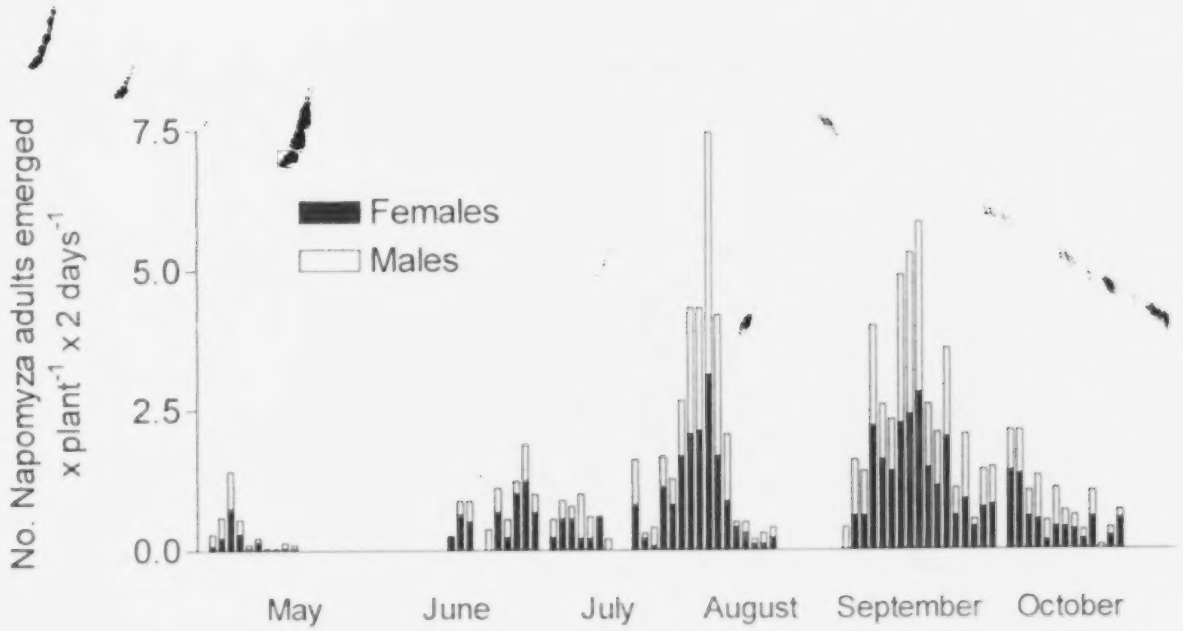

Figure 22 Emergence of adult *Napomyza* sp. near *lateralis* from scentless chamomile in 2000 (n = 1,868). CABI Bioscience, Delémont Switzerland.

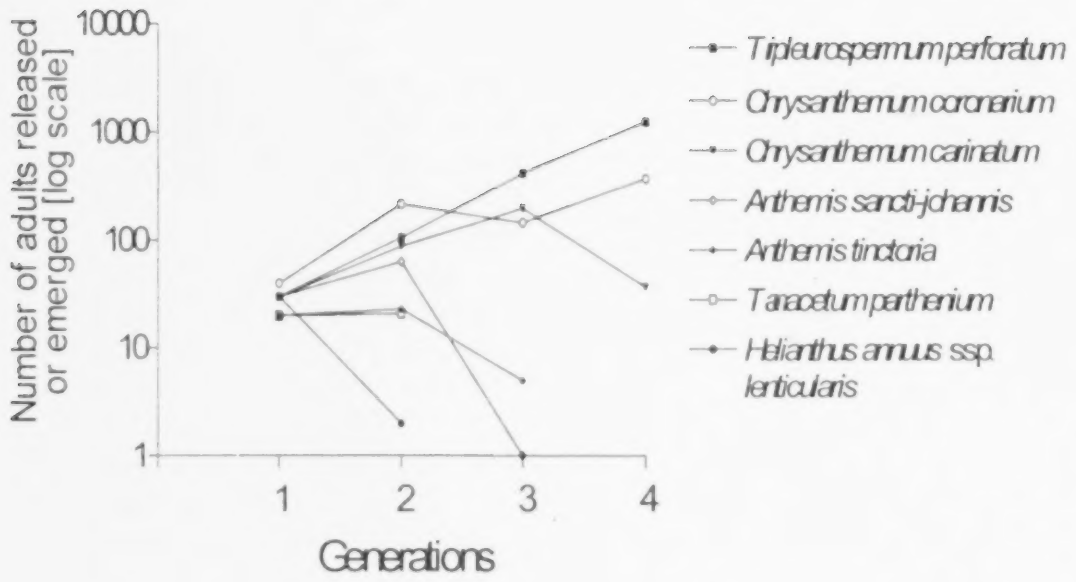

Figure 23 Numbers of adult *Napomyza* sp. near *lateralis* emerging in successive generations on scentless chamomile (*Tripleurospermum perforatum*) and other test plants. "Continuation trial", CABI Bioscience, Delémont, Switzerland, 2000.

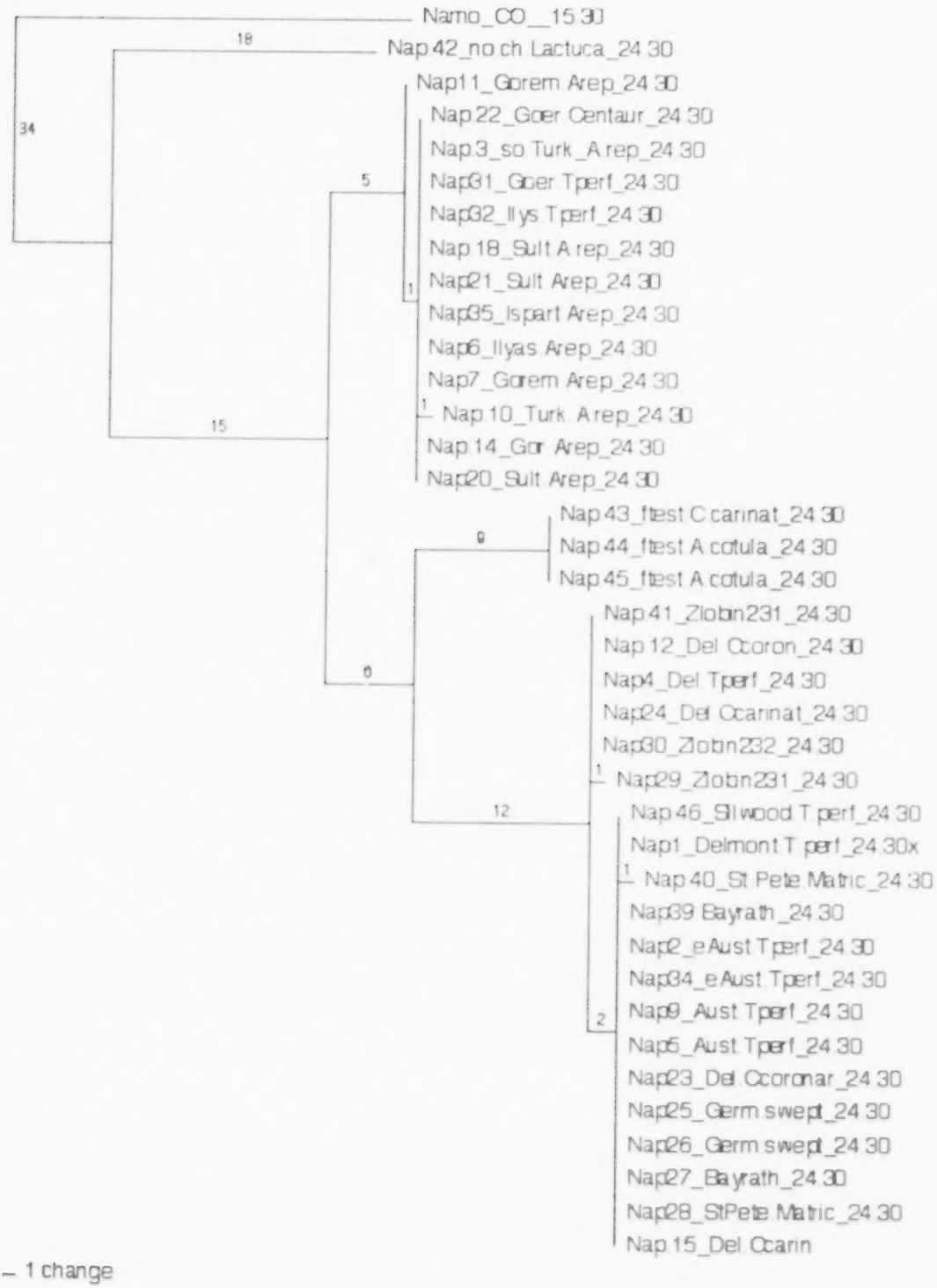

Figure 24 Relationships of *Napomyza* sp. near *lateralis* individuals reared from scentless chamomile, Russian knapweed, and other hosts in Europe, from mitochondrial COI sequences. S. Scheffer, USDA.